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Kentucky Water Resources Research Institute Annual Technical Report FY 2012

Introduction

The 2012 Annual Technical Report for Kentucky consolidates reporting requirements for the Section 104(b) base grant award into a single document that includes; 1) a synopsis of each research project that was conducted during the period, 2) citations for related publications, reports, and presentations, 3) a description of information transfer activities, 4) a summary of student support during the reporting period, and 5) notable awards and achievements during the year.

Research Program Introduction

The activities supported by the Section 104(b) program funds and required matching are interwoven into the overall program of the Kentucky Water Resources Research Institute. Additional research, service, and technology transfer activities were funded through a variety of other sponsors. Memoranda of Agreement projects with the Kentucky Division of Water included 1) Total Maximum Daily Load development for several Kentucky streams and 2) Nutrient Management Stakeholder Engagement in the Floyds Fork watershed near Louisville. The Kentucky River Authority supported watershed management services in the Kentucky River basin and a small grant program to fund local grassroots organizations. The National Institute of Environmental Health Sciences supported research translation activities through the Superfund Public Outreach Program. The Department for Hometown Security funded two projects related to security for water infrastructure. The Kentucky Department for Environmental Protection supported 3 students through an Environmental Protection Scholarship Program coordinated by the Institute. The Division for Compliance Assistance funded support through the KWRRI for all MS4 communities to send a representative to the annual meeting of the Kentucky Stormwater Association in August 2012 and to an MS4 Monitoring Plan Workshop in November.

Eleven student research enhancement projects were selected for support through 104(b) FY2012 funding. A previous project (2011KY167B) was revised and resubmitted with a new principal investigator through a different lead institution in early 2012 (project ultimately funded is 2011KY213B). Due to an extremely late start, these efforts were combined with Project 2012KY201B and a single project report is provided for those efforts. Projects funded in 2012 were conducted at the University of Kentucky (5), Morehead State University (1, in conjunction with Asbury University), Murray State University (1), Eastern Kentucky University (2), and Western Kentucky University (2). Projects represented a variety of discipline areas including biology (4), plant and soil science (1), geology (3), chemistry (1), biosystems and agricultural engineering (1), and public and environmental health (1). The goal of this approach is to support a number of student-based efforts representing a variety of discipline areas at numerous educational institutions throughout the state to develop broad research capacity related to water resources. Many state environmental agencies are experiencing a significant loss of personnel through retirements and it is critical that undergraduate and graduate students are well trained and available to help fill this void. Reports for three 2011 projects that were completed in FY2012 (2011KY169B, 2011KY174B, and 2011KY213B) are included in this report. These are followed by reports for the eleven 2012 student research enhancement projects.

Delineating solute inputs to the headwaters portion of the Cane Run/Royal Spring basin

Basic Information

Title:	Delineating solute inputs to the headwaters portion of the Cane Run/Royal Spring basin
Project Number:	2011KY169B
Start Date:	3/1/2011
End Date:	12/31/2012
Funding Source:	104B
Congressional District:	KY 6th
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Solute Transport, Water Quality
Descriptors:	karst, nutrients, isotopic trends
Principal Investigators:	Alan Fryar, James Dinger

Publications

1. Skees, Catherine F. and Alan E. Fryar, 2012, Delineation of solute inputs to the headwaters portion of the Cane Run/Royal Spring basin, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p. 83.
2. Skees, Catherine F. and Alan E. Fryar, 2012, Using geochemical analyses to delineate solute inputs to the headwaters portion of the Cane Run/Royal Spring basin of north central Kentucky, Geological Society of America Abstracts with Programs, Boulder, CO, vol. 44, no. 5.

Delineating Solute Inputs to the Headwaters Portion of the Cane Run/Royal Spring Basin

Problem and Research Objectives

The Cane Run watershed and underlying karst aquifer, which discharges to Royal Spring, serve as the primary source of drinking water for the city of Georgetown, Kentucky. The headwaters of this basin are located in north Lexington, an urban area with mixed industrial and residential land uses. The water quality of the basin has been compromised by non-point-source pollutants, including bacteria, sediment, and nutrients. The Lexington-Fayette Urban County Government signed a consent decree with the U.S. Environmental Protection Agency to address combined sanitary-storm sewer overflows in 2008. Other potential sources of pollution include the University of Kentucky (UK) Spindletop Farm, the Kentucky Horse Park, private farms, and a trailer park. Current monitoring is limited to bacteria at several sites, plus analyses of Royal Spring water by the Georgetown Municipal Water and Sewer Service for constituents regulated under the Safe Drinking Water Act. General variations in groundwater quality throughout the basin have not been thoroughly investigated. Delineating solute inputs would aid in the development of remedial and future water-quality monitoring efforts.

For the duration of one year, water samples were collected for analyses of major anions, nutrients (nitrate, nitrite, ammonium, and phosphate), stable isotopes, and field parameters (temperature, specific conductance, pH, and dissolved oxygen) within the urbanized headwaters. We expect that field parameters, solutes, and stable isotopes can provide evidence of sources of anthropogenic recharge. Such data can theoretically help identify areas of groundwater/surface water interaction and solute inputs, including nutrient hotspots. Chloride and stable isotopes are considered to be conservative groundwater tracers, i.e., not affected by chemical reactions (other than dissolution, in the case of chloride). Stable isotopes are differentially fractionated by evaporation, and thus should indicate industrial cooling-water inputs. Understanding the processes affecting water quality within the Cane Run headwaters would enhance community and governmental efforts to protect this and similar karst aquifers.

Methodology

Nutrients, other anions, and stable isotopes of water were monitored biweekly from September 7, 2011, to August 22, 2012, at seven sampling sites. These include two springs (the Citation Estavelle [CE] and Highland Spring [HS]) and five sites along surface drainages (CR1, CR4, CR5, CR6, and CR14) (Fig. 1). CR5 was monitored during two storm events at half-hour to hourly intervals in June and September 2012 using an ISCO automated sampler. CR5 was chosen because of perennial flow, accessibility and proximity to an area of residential and commercial development. Precipitation samples (collected since June 2011) were analyzed for stable isotopes of water. Isotopic data collected within the headwaters were compared to a meteoric water line for the Fayette/Scott county area. Temperature, specific conductance, and pH were measured in the field using a YSI multiparameter probe. Dissolved oxygen was measured by Winkler titration within 8 hours of sampling. Samples for lab analyses were passed through 0.45-micron pore-size disposable filters. Samples were analyzed for sulfate, chloride, and phosphate by ion chromatography in the UK Environmental Research and Training Laboratory, and for nitrate and ammonium in the UK Department of Forestry. Oxygen-18 and hydrogen-2

(deuterium) were analyzed in the UK Department of Earth and Environmental Sciences stable isotope laboratory using a gas-source, continuous-flow, isotope-ratio mass spectrometer.

Networked computers and software, including ArcGIS and MATLAB, were used for data analysis and interpretation. Data were analyzed by standard graphical techniques (e.g., bivariate plots). Analytical results were entered into a GIS coverage of the basin. Layers include soils, lithology, watershed boundaries, streams, active U.S. Geological Survey (USGS) stream gages, roads, counties, sinkholes, and aerial imagery, as well as digital elevation model and land cover designations. Spatial and temporal trends in solute and isotope concentrations were statistically interpreted, as well as relationships between concentrations and hydrologic parameters (e.g., precipitation and stream stage, which were monitored at several sites in the basin).

Principal Findings and Significance

Results allow us to infer trends and anomalies for the different sampling locales under different conditions. Field water-quality data show significant variation between wet conditions (antecedent rainfall > 1.9 cm within 72 hours prior to sample collection) and normal/baseflow conditions (antecedent rainfall < 1.9 cm) as well as seasonal variations. The threshold of 1.9 cm was chosen based on average rainfall accumulations during the 1-year study. Field parameters are least variable among the sites that are considered to be dominantly groundwater (CE and HS). In fact, CE appears to be a spring rather than an estavelle (an orifice that functions as a discharge point at low flow and a recharge sinkhole at high flow). CR1, located within the most industrialized area of the headwaters, exhibits the greatest variations in both field and lab analytes. Concentrations of chloride, sulfate, and nutrients are elevated under normal/baseflow conditions relative to wet conditions (Fig. 2). The temperature variations of this site could be indicative of wastewater input from the surrounding industrial area. Samples obtained from the CR1 site are isotopically heavier than the other surface water sites within the headwaters (Fig. 3). Nutrient concentrations are highest in the residential locations, including CR4 and HS. These sites have higher levels of phosphate and nitrate under both wet and dry conditions, especially in winter. Leakage from sanitary sewers could result in elevated nutrient concentrations under baseflow conditions. Nutrient and field data for CR1 data indicate dilution following storms.

During the June 2012 storm, samples were collected every half hour during the first 12 hours and hourly samples were collected subsequently from 8 am June 1 to 8 am June 2. Rainfall accumulation from May 31 to June 2 totaled 1.1 cm. Discharge data for this storm were not obtained because the USGS gauge downstream on Berea Road was relocated to Citation Boulevard during spring–early summer. Sulfate and chloride concentrations dropped as rainfall increased, indicating dilution. Conversely, as precipitation increased, nitrate and phosphate concentrations increased. Phosphate spiked immediately following the most intense rain period, then became more diluted like sulfate and chloride. After the 12th hour, isotopic values became more positive, suggesting a relatively rapid recession toward baseflow as rainfall tapered off.

The storm event sampled in September 2012 had a total rainfall accumulation of 1.3 cm. Hourly samples (46 total) were collected from 1 pm September 17 to 11 am September 19. Discharge records for this storm were available for the USGS gauge located on Cane Run at Citation Boulevard, less than a mile south of the CR5 tributary. The hydrograph shows a 4-hour

response time of the gauge to the storm. The peak precipitation was 0.51 cm and the peak discharge was 1.35 cubic feet per second ($0.0382 \text{ m}^3/\text{second}$). The discharge records seem to indicate baseflow following this storm event was not attained until after September 19, when sampling ceased. The isotopic trends again indicated a relatively quick response to changes in precipitation, with slightly more positive values after the 12th hour, 4 hours after the storm's peak. However, the isotopic response was subdued compared to the early summer storm, which suggests that stream flow was higher immediately prior to the initial rainfall of the September storm. Precipitation records for spring and summer months of 2012 support this inference.

Results of this study suggest that nutrient levels within the headwaters are low compared to results from the more agricultural areas of the watershed. Sulfate and chloride seem to be highest at the more industrial sites, specifically CR1 and CR14. The more residential and agricultural headwater sites, CR4 and HS, were expected to show heightened nutrient levels following precipitation events. However, nitrate levels at these sites, while slightly higher following storm events, never exceeded the maximum contamination limit (MCL) set by the EPA (10 mg/L as N). Phosphate was also found below the MCL, and values were near the method detection limit in most sampling events within the headwaters. Isotopic anomalies at CR1 could indicate periodic wastewater discharge from surrounding industries.

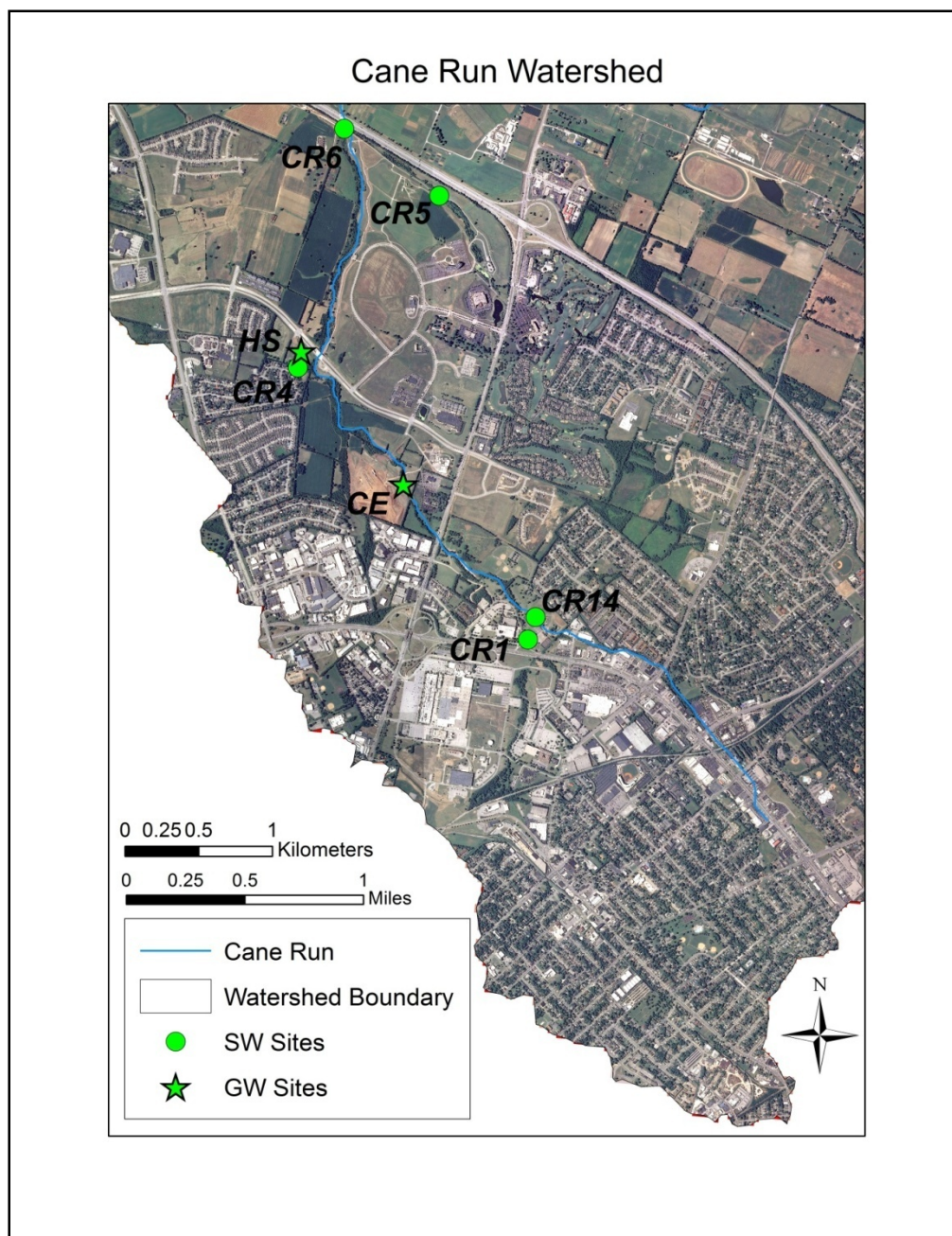


Fig. 1. Cane Run headwaters sampling locations.

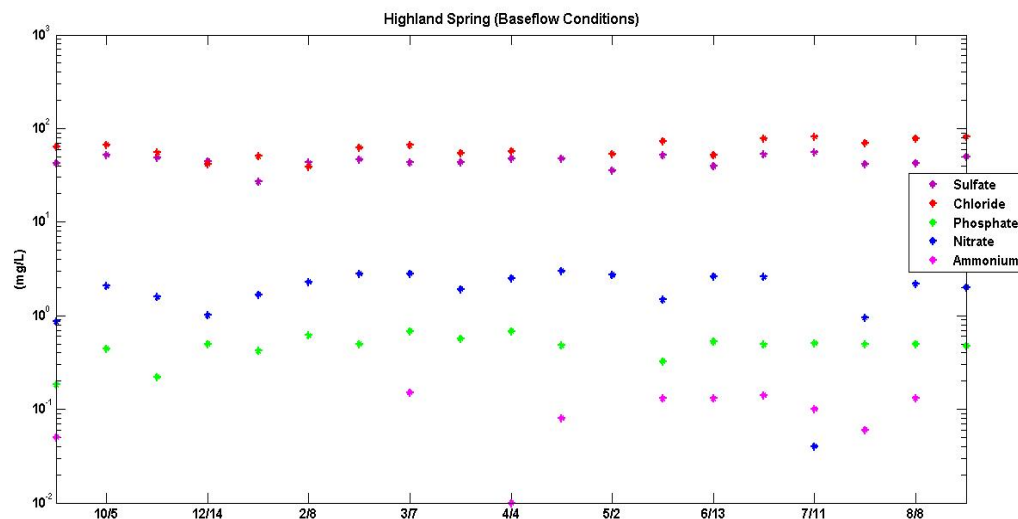


Fig. 2. Highland Spring anion concentrations under baseflow conditions (note that ammonium values below the method detection limit were not plotted).

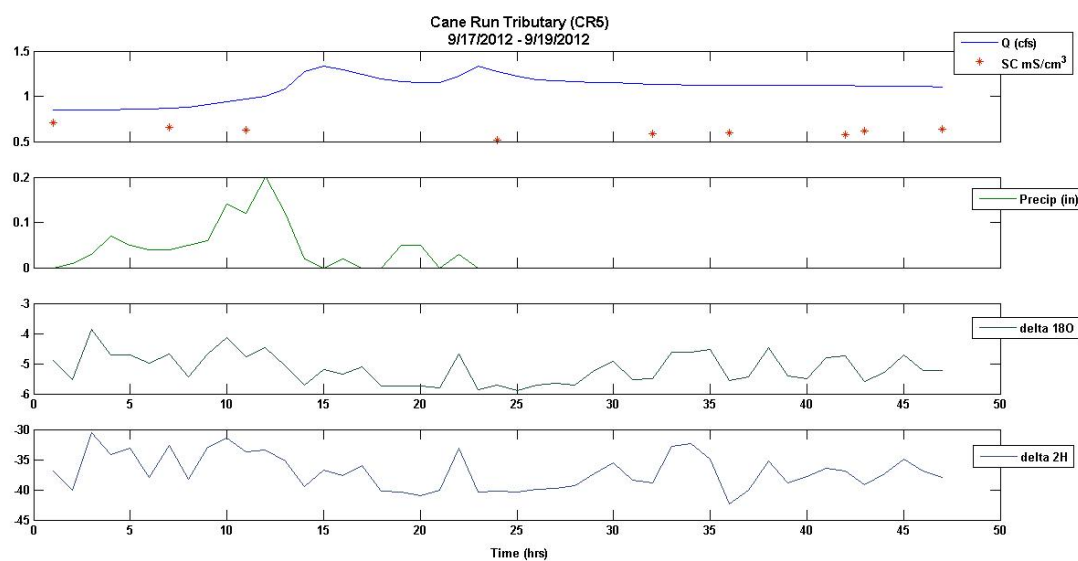


Fig. 3. CR5 response to 1.3 cm of rainfall in September 2012 ($\delta^{18}\text{O}$ and $\delta^2\text{H}$ are shown in ‰ values relative to the V-SMOW standard).

By-proxy monitoring of aqueous nitrate photolysis and the effect of hydroxyl radical

Basic Information

Title:	By-proxy monitoring of aqueous nitrate photolysis and the effect of hydroxyl radical
Project Number:	2011KY174B
Start Date:	3/1/2011
End Date:	12/31/2012
Funding Source:	104B
Congressional District:	KY 2nd
Research Category:	Water Quality
Focus Category:	Geochemical Processes, Nitrate Contamination, Methods
Descriptors:	infrared spectra, FTIR spectroscopy, atmospheric contaminants
Principal Investigators:	Matthew Nee

Publications

1. Wyatt, Jonathan and Matthew Nee, 2012, By-proxy monitoring of aqueous nitrate photolysis and the effect of hydroxyl radical, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p. 87.
2. Barnes, Garrett and Matthew Nee, 2012, The Effect of Ionic Strength on Symmetry Breaking in Aqueous Nitrate Ion, in Proceedings 2012 Kentucky Academy of Sciences Annual Meeting, Richmond, KY
3. Nee, Matthew, 2013, Thermal Dependence of Solvation Geometries in Aqueous Nitrate Ion Solutions, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 55-56

By-Proxy Monitoring of Aqueous Nitrate Photolysis and the Effect of Hydroxyl Radical

Problem and Research Objectives

The photolysis of aqueous nitrate ion (NO_3^-) in the natural environment has been shown to produce a variety of compounds which are considered either human health hazards or are potentially environmentally dangerous. These include the nitrogen oxides ($\text{NO}_x = \text{NO} + \text{NO}_2$) and ozone (O_3). To properly model the impact that nitrate photolysis has on climate change and atmospheric composition, a full set of kinetic data (rate constants, mechanisms, and branching ratios) must be known for the complex network of reactions which terminate in the production of NO_x and O_3 . The laboratory experiments performed here represent one aspect of the information which is necessary for proper modeling of the Earth's atmosphere. The original project objective was to use the broken symmetry in the infrared spectrum of nitrate ion as a proxy for the reaction kinetics occurring surrounding the chromophore. We have shown that no change is indicated by our experiments in the infrared spectrum. However, in the process, we have found that we are able to observe a temperature dependence to the split peaks in the infrared spectrum of nitrate ion. This information gives fundamental insight into the energetic origin of the two peaks and allows us to explore symmetry breaking in nitrate ion as we search for other means of exploring the kinetics following photolysis.

Methodology

Infrared spectra of nitrate ion were collected every 30 seconds for a total of three and a half hours following exposure to ultraviolet radiation (photolysis). A standard mercury lamp source was used as the photolysis radiation. The infrared spectrometer used was a HP Spectrum One Fourier transform infrared (FTIR) spectrometer. Samples were sealed between two sapphire windows in a custom-built temperature-controlled sample cell holder. Sapphire is needed because it is resistant to degradation by water and also passes ultraviolet radiation. Normally, aqueous spectra cannot be well monitored with infrared spectroscopy because of the large signals due to water absorption. However, NO_3^- has its primary infrared signal (asymmetric stretching mode) in the range from 1200 to 1500 cm^{-1} , which is completely clear of any water absorption. It is well established that the spectral line shape is described by two Gaussian peaks corresponding to different molecular geometries of nitrate which exist in solution. Because these two geometries arise as a result of the electrostatic forces between nitrate ion and water molecules, our original hypothesis was that, as the photolysis reaction proceeded and the concentrations of different components changed, the relative amounts of the two geometries would change. This would manifest as a change in the relative areas of the Gaussians used to fit the infrared spectrum.

Originally, we had intended to perform these experiments in the presence of benzoic acid, which has been shown to serve as a proxy for the presence of hydroxyl radical, one of the main reactants responsible for the production of NO_x following nitrate ion photolysis. Although we were not able to see any changes to the nitrated ion signal, those experiments did reveal for the first time that the infrared spectrum may be sufficient to indicate the presence of hydroxyl radical in solution using the benzoic acid proxy.

Principal Findings and Significance

Figure 1 shows the complete spectra for several representative times following photolysis. Both show strong water signals which are saturated (despite background subtraction). These are the “noisy” sections of the spectrum from 500 to 1000 cm^{-1} , from 1500 to 1650 cm^{-1} , and from 3000 to 3650 cm^{-1} . Also prominent in both is the nitrate ion asymmetric stretch (1200 to 1500 cm^{-1}), which will be discussed in greater detail below. The primary difference seen between these two spectra lies in the region from 2000 to 2400 cm^{-1} . In this region, circled in Figure 1, the spectra evolve slowly over time for the spectra in which benzoic acid has been added as a proxy for the detection of hydroxyl radical. Thus, the amount of hydroxyl radical present can be assumed to evolve with time following photolysis.

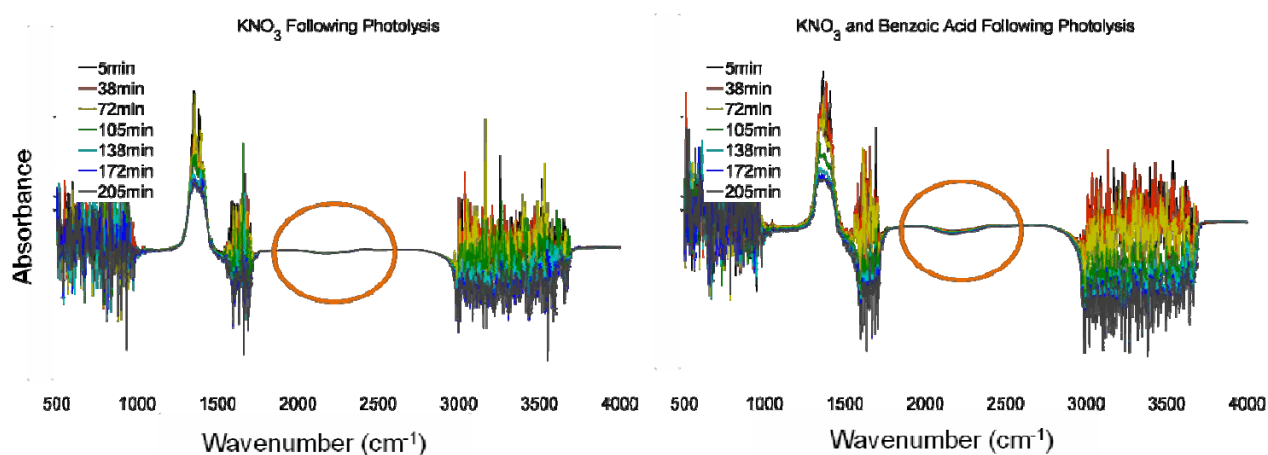


Figure 1. Representative FTIR spectra of solutions of KNO₃ with and without the addition of benzoic acid as a proxy for the generation of hydroxyl radical. On the right, changes to the intensity of the broad feature from 2000 to 2400 cm^{-1} are interpreted to indicate changes in the presence of hydroxyl radical.

The results of our experiments with and without benzoic acid are shown in greater detail for the asymmetric stretching region in Figure 2. These results show a representative sample of spectra collected at various times following five minutes of UV exposure. In the top panel, several spectra are shown. Although an overall total decrease in nitrate ion signal appears to occur during the course of the experiment, the relative intensities of the peaks does not appear to change. This is illustrated in the lower panels, which show the peak areas fitted from all data collected. To support that the peak fitting algorithm used is valid, the centers of the fitted peaks are also shown. It is clear that neither the areas nor the centers of the peaks change during the course of the reaction. Thus, while we are able to monitor the presence of hydroxyl radical by adding benzoic acid, we are not able to correlate this to a change in the nitrate peak areas as we had originally hoped.

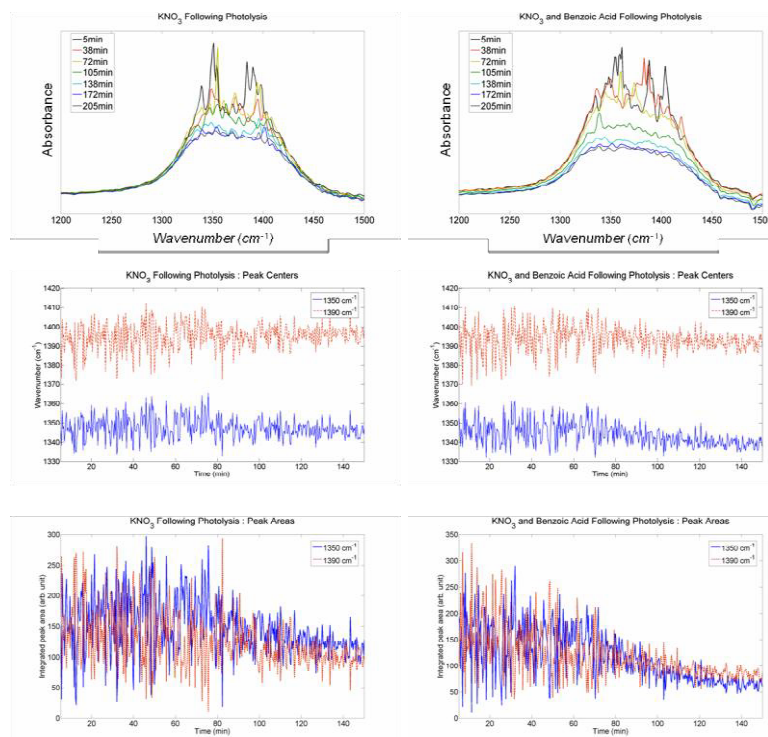


Figure 2. Details of the asymmetric stretch region following photolysis. Solutions with (right) and without (left) benzoic acid behaved indistinguishably.

Beyond our original intentions, we have shown that the *relative intensities* of the different solvent geometries *does* change as a function of temperature, in the presence of photolysis. This behavior is shown in Figure 3. As the temperature increases, the peak at high frequency increases as the peak at low frequency decreases in area. This implies that, as more thermal energy is available to the system, the solvation geometry with the higher frequency becomes accessible.

This project made good progress in spite of some minor hurdles regarding formal staffing. While the original Co-PI was not able to take advantage of this funding (left our program), an undergraduate was funded to continue the project during January and February. We completed milestone (1) from our original plan. This has allowed us to conclude that, while our original hypothesis was not correct, FTIR spectroscopy can be used to monitor hydroxyl radical by proxy in the presence of benzoic acid. This will prove useful to future studies which need the sensitivity and selectivity that FTIR provides to monitor other reactions in aqueous solution. All of this work will continue to improve our understanding of the production of atmospheric contaminants such as NO_x and O_3 .

A student enrolled in our combined BS/MS program and another supported through our department's NSF-REU program continued the data collection for the temperature-dependent studies. He collected higher-quality temperature dependent data (also shown in Figure 3) which allowed us to determine the thermodynamic parameters relevant to the different solvation geometries in aqueous nitrate. The dependence of this data on ionic strength is particularly promising, and has led to the development of new hypotheses. From a more fundamental standpoint, we are poised to explore a completely different aspect: the origin of the two peaks seen in the nitrate ion asymmetric stretch spectrum.

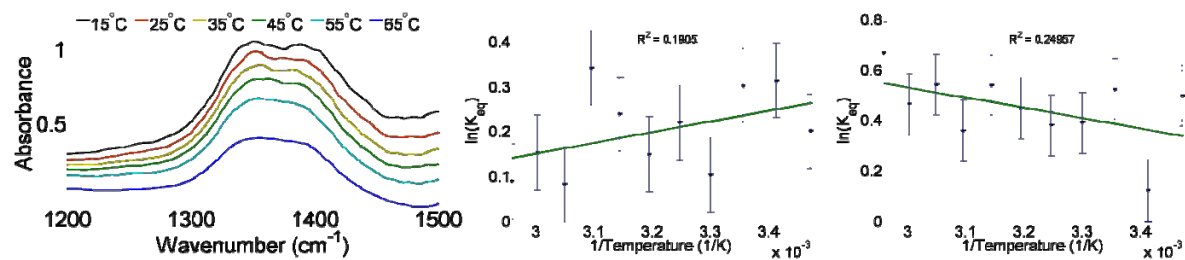


Figure 3. Left: FTIR spectra of 0.2-M KNO₃ solutions at various temperatures. The lineshape shifts towards more absorbance at 1390 cm⁻¹ at higher temperatures.

Center: van't Hoff plot using the peak area ratios for K_{eq} . From Eq. 1, the slope and intercept reveal the enthalpy and entropy of the conversion reaction for going between different solvation geometries.

Right: For higher ionic strength solution, the trend with temperature reverses.

Use of gene expression in longear sunfish (Lepomis megalotis) and green sunfish (Lepomis cyanellus) as a biomarker of polychlorinated biphenyl and metal exposure

Basic Information

Title:	Use of gene expression in longear sunfish (Lepomis megalotis) and green sunfish (Lepomis cyanellus) as a biomarker of polychlorinated biphenyl and metal exposure
Project Number:	2011KY213B
Start Date:	1/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 5th and 6th
Research Category:	Biological Sciences
Focus Category:	Toxic Substances, Water Quality, Surface Water
Descriptors:	endocrine active compounds, water quality monitoring
Principal Investigators:	David Peyton, Ben Brammell

Publications

1. Johnson, Matthew, Ben Brammell, David Peyton, Ben Adams, and Andrew Wigginton, 2013, Gene Expression in Sunfish as a Biomarker of Contaminant Exposure, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 85
2. Johnson, Matthew, Ben Brammell, David Peyton, Ben Adams, and Andrew Wigginton, 2012, Gene Expression in Sunfish as a Biomarker of Contaminant Exposure, Kentucky Academy of Science Annual Meeting October 19-20, 2012, Eastern Kentucky University, Richmond, KY
3. Kirtland, Marina, Meredith Eckstein, David Peyton, Ben Brammell, Andrew Wigginton, and Scott Lynn, 2012, Gene Expression in Native Sunfish as a Biomarker of Contaminant Exposure, Kentucky Academy of Science Annual Meeting October 19-20, 2012, Eastern Kentucky University, Richmond, KY
4. Kirtland, Marina, Meredith Eckstein, David Peyton, Ben Brammell, Andrew Wigginton, and Scott Lynn, 2012, Gene Expression in Native Sunfish as a Biomarker of Contaminant Exposure, Southern Regional Honors Conference, April 4-6, 2012, Louisville, KY
5. Johnson, Matthew, 2013, Use of Biomarker Gene Expression in Contaminant Detection, Annual Undergraduate Research Presentation, Asbury University, Wilmore, KY

Use of Gene Expression in Longear Sunfish (*Lepomis megalotis*) and Green Sunfish (*Lepomis cyanellus*) as a Biomarker of Polychlorinated Biphenyl and Metal Exposure

Problem and Research Objectives

Recent studies have described widespread incidences of anatomical and physiological alterations in fish linked to waterborne pollutants (1, 2; 3, 4). Observed alterations include occurrence of intersex fish (5), abnormal hormone levels (6), reduction in reproductive success (7), the presence of tumors (8), and abnormal liver to body weight ratios (9). These alterations have been demonstrated to result from pollutants that affect the hypothalamus-pituitary-gonad axis, collectively referred to as endocrine active compounds (EACs) (10). EACs include a number of prevalent water borne pollutants including organochlorine pesticides, polychlorinated biphenyls (PCBs), heavy metals, pharmaceuticals, and surfactants (5). Currently the effects of EACs in freshwater fish are most frequently observed through studies examining histology of the gonads (5), alterations in plasma hormone levels (11), or detailed studies examining fecundity and reproductive success (6). Although these studies provide great insight into the nature of contaminant effects they are expensive, time consuming, and require specialized techniques. Many contaminants interact not only with the endocrine system but also with other aspects of normal physiology, resulting in alterations of specific enzymes or proteins known as biomarkers (12). Monitoring the expression of biomarkers provides an alternative mechanism of contaminant detection that offers a number of advantages (12, 13, 14, 15, 16, 17).

Pollutants found in surface waters have frequently been observed to alter the health and reproductive status of resident fish populations (1, 2, 3; 4, 5). Intersex fish, a condition frequently attributed to contaminant presence, have been observed in Europe for over a decade (2) but recent studies reporting widespread observations of intersex fish in North America (5) highlight the need for a greater understanding of this phenomenon. Many contaminants interact not only with the endocrine system but also with other aspects of normal physiology, resulting in alterations of specific enzymes or other proteins known as biomarkers (12). The use of biomarkers in pollutant detection offers a number of advantages including the ability to quickly screen for a wide variety of compounds, the ability to detect contaminants of an intermittent nature, and the relatively limited skill set and facilities necessary to utilize biomarkers. In addition, advances in biotechnology have greatly enhanced both the sensitivity and convenience of quantifying biomarkers at the level of mRNA expression. Despite these clear advantages to biomarker utilization in contaminant detection, few studies have utilized biomarker expression in native North American fish species that actually reside in contaminated waters. This study supported development of tools necessary to utilize eight biomarkers in two widely distributed fish species using real-time quantitative PCR. The collection site for this project has highly characterized contaminant levels and has been the location of multiple previous studies utilizing similar biomarkers in a well-characterized model (15, 21). Therefore, at the completion of this project, a large volume of pre-existing data will facilitate a thorough evaluation of gene-expression analysis as a method of contaminant detection and monitoring.

Previous work has provided a well documented distribution of metal and PCB contamination at the sites to be used in the proposed study (18, 19, 20). In addition, two recently conducted studies examined alterations in a number of biomarker genes in zebrafish (*Danio*

rerio), demonstrating the bioavailability of contaminants in these sites. One study conducted in the spring of 2009 used water collected from the same sites that will be utilized in the proposed study and exposed zebrafish to this water in the laboratory. Both catalase and cytochrome P4501A (CYP1A) were altered in contaminated sites, and the CYP1A response seemed to be a direct consequence of the presence of polychlorinated biphenyls (15). A study conducted in the fall of 2010 exposed caged zebrafish to water in the same sites, again documenting the presence of elevated CYP1A expression correlating to PCB contamination (22). The current study provides a unique opportunity to examine expression of biomarkers in two previously uncharacterized species in a system in which both contaminant levels and biomarker response in a model organism have been recently documented. While using biomarkers in model organisms such as zebrafish can be an effective method, it necessitates complex studies whereby the organisms are either exposed in cages or water from the sites is returned to the lab. Examination of biomarkers in widely distributed native fish increases the ease with which biomarker studies may be conducted and provides valuable information on the effects of contaminants on native organisms. The results of this study will facilitate the use of biomarkers in contaminant detection studies by providing tools and data necessary to use common fish to detect contaminants.

We examined the effect of contaminant exposure on three biomarkers at the level of mRNA expression in a species of sunfish (green sunfish, *Lepomis cyanellus*) widely distributed throughout North America. The results of this study provide data essential to the utilization of biomarkers for contaminant detection using native species and in addition will provide tools, in the form of nucleic acid sequences, that are universally available to researchers and enable the widespread use of biomarkers for contaminant detection.

The specific objectives of this research were to:

1. Acquire nucleic acid sequences from green and longear sunfish that represent several established biomarker genes known to be altered in fish following exposure to various contaminants: cytochrome P4501A, metallothionein, and glutathione S-transferase (2, 12).
2. Utilize these sequences to quantify biomarker gene expression in fish collected in both reference areas and areas receiving industrial effluent (and known to contain relatively high levels of both organic and metal contaminants).

Methodology

Study Site - The Paducah Gaseous Diffusion Plant (PGDP) is a uranium enrichment facility located in western KY. The streams surrounding the PGDP have a long and well documented history of contamination by metals and PCBs and both longear sunfish and green sunfish, the focus of this study, are abundant in these watersheds (18,19). In addition, recent studies document the presence and bioavailability of both PCB and metal contaminants in these streams (15).

Field collections - Longear and green sunfish were collected from both contaminated and reference sites surrounding the PGDP. Gill and liver tissue were removed and flash frozen in liquid nitrogen for transport back to the lab.

RNA extraction and cDNA generation - Liver tissue was removed from storage in the lab and a subsample removed and transferred to Ambion's lysis buffer for total RNA isolation. First strand cDNA was generated using Applied Biosystem's High Capacity RNA-to-cDNA Kit according to the manufacturer's instructions.

Gene sequencing - RACE PCR was used to amplify segments of cDNA that was subsequently checked using gel electrophoresis for expected length. Candidate fragments were ligated to the P-GEM-T Easy vector (Promega, Inc.) and transformed into JM-109 *E. coli* cells. Primers for real-time PCR were designed for expression analysis.

Real-time PCR - Quantification of mRNA in the livers was conducted by real-time PCR using the iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc., Tokyo, Japan) and iCycler iQTM Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.).

Principal Findings and Significance

The sequence of three genes frequently utilized as indicators of contaminant exposure were obtained (Table 1 and 2, Figure 3). Partial coding sequences for sequences for cytochrome P4501A, metallothionein, and glutathione S-transferase were obtained for green sunfish (*Lepomis cyanellus*) and metallothionein and glutathione S-transferase were obtained for longear sunfish (*Lepomis megalotis*). The sequences are shown in Tables 1 and 2. The acquisition of these sequences makes the utilization of these commonly used biomarkers possible in gene expression studies in both of these fish species found throughout most of North America.

Gene expression was quantified in green sunfish collected in clean and contaminated sites around the PGDP in western Kentucky (Figure 1). Relative expression of GST was greatest at BC8 and lowest at BC1A (the reference location), however, no significant differences were observed between sites. Gene expression of MT was lowest at BC5 and relatively high at BC1A although again, no significant differences were observed between sites. CYP1A gene expression was greatest at BC5 and lowest at BC1A with no significant differences observed.

Several studies collectively support the conclusion that *Lepomis* species may be relatively insensitive to polychlorinated biphenyls and have an inherent resistance to induction of CYP1A and other pollutant sensitive genes, rather than an acquired resistance as has been frequently observed in other species. In this study, there were no significant differences in green sunfish (*Lepomis cyanellus*) gene expression (GST, MT, and CYP1A) between the contaminated and reference sites. A similar study (15) found no significant difference in gene expression between reference and contaminated sites were observed in four of six pollutant sensitive genes examined in zebrafish (*Danio rerio*) caged in waters surrounding the Paducah Gaseous Diffusion Plant. However, that study did differ from this one in that significant differences were observed in CYP1A expression in caged zebrafish. This difference is perhaps reflective of the reported limited sensitivity of *Lepomis* species to PCB type inducers (16). This study provides data essential to evaluating the response of native fish to contaminants found in their environment as well as information perhaps relevant to the trophic transfer of contaminants from aquatic to terrestrial systems resulting from fish able to persist in relatively high contaminant levels.

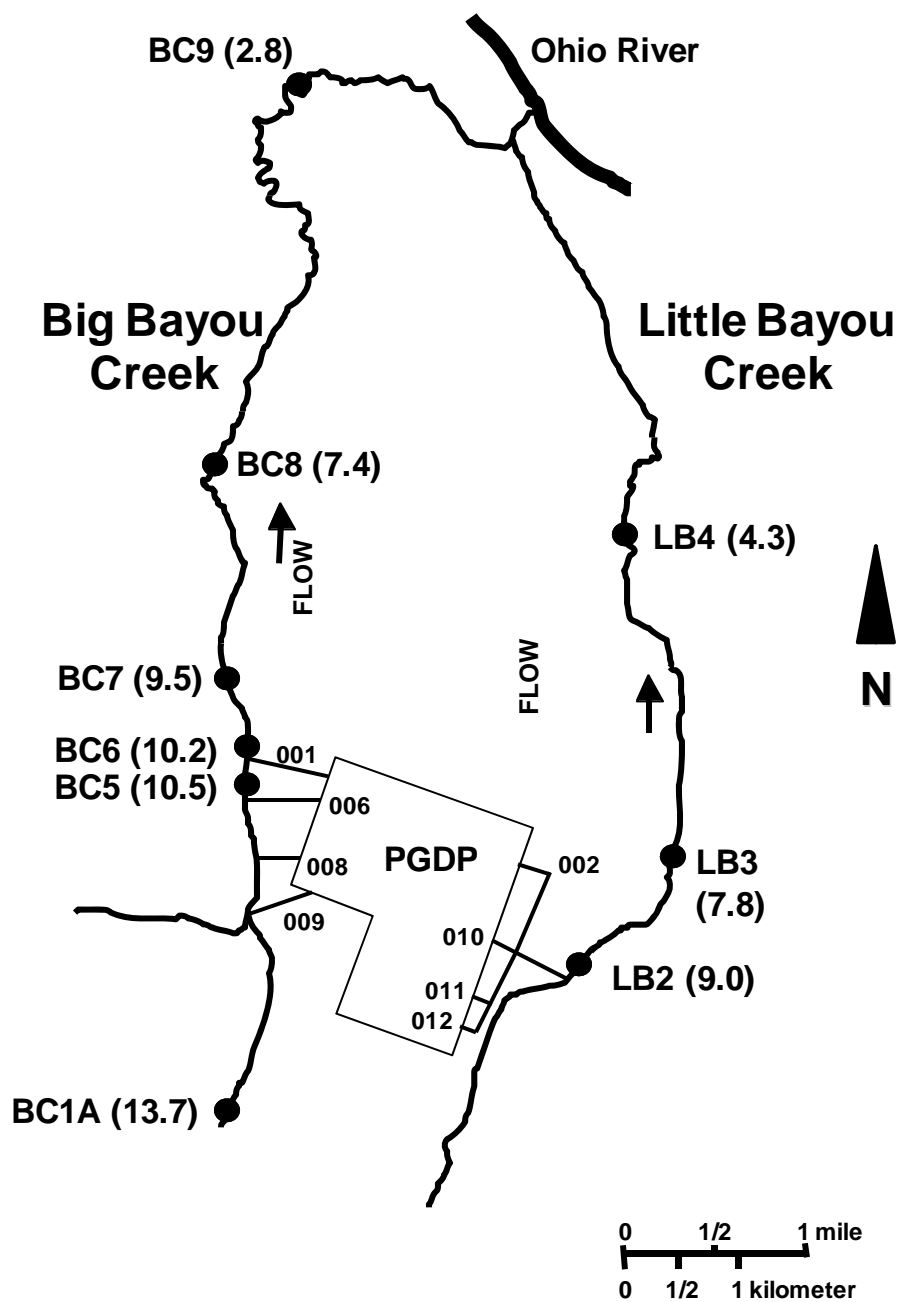


Figure 1. Map of study sites surrounding the Paducah Gaseous Diffusion Plant located in Western Kentucky.

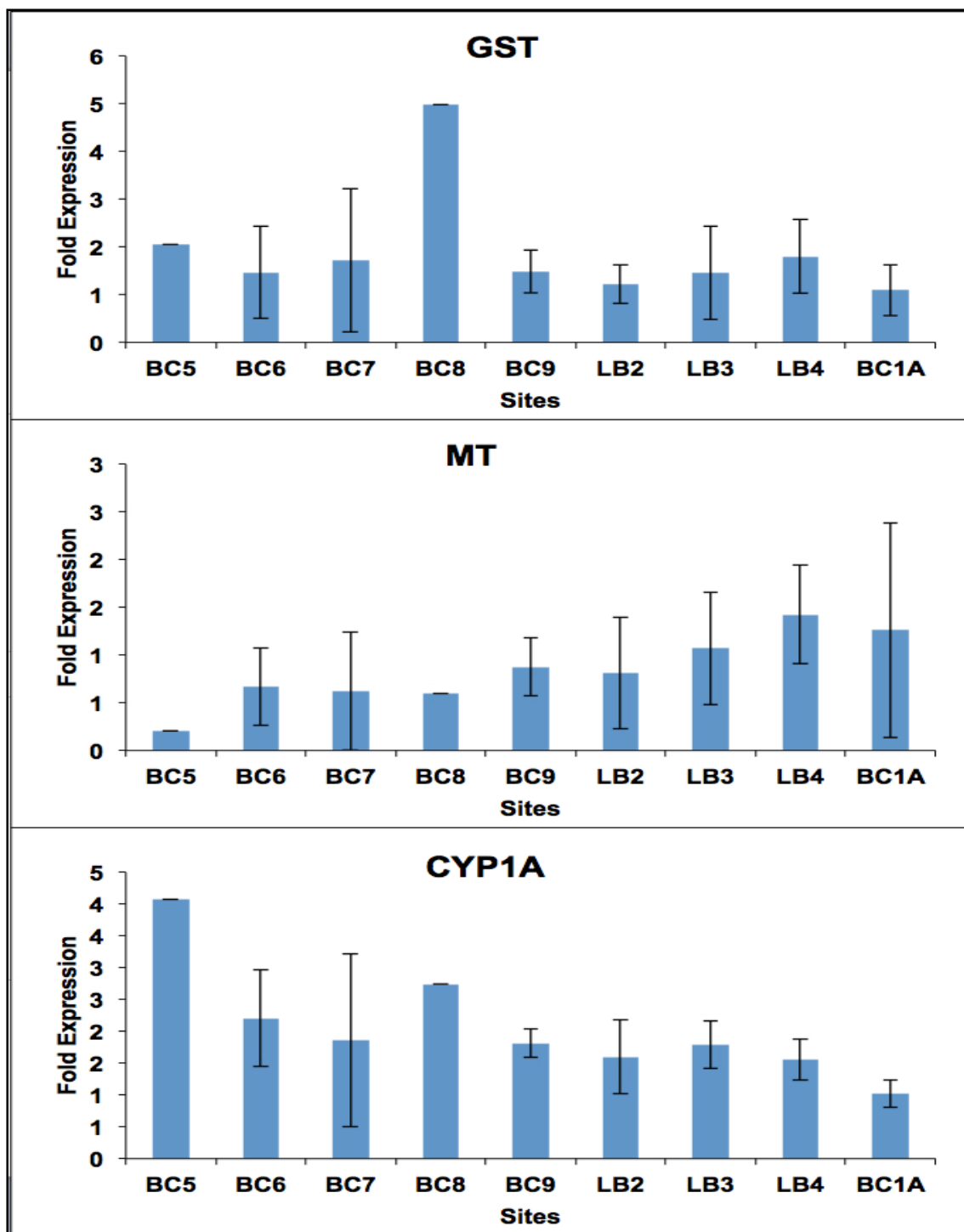


Figure 2. Gene expression (glutathione S-transferase (GST), cytochrome P4501A (CYP1A), and metallothionein (MT) in green sunfish (*Lepomis cyanellus*) collected from Bayou Creek (BC) and Little Bayou Creek (LB) around the Paducah Gaseous Diffusion Plant in Paducah, KY (McCracken County).

A. Score = 123 bits (136), Expect = 2e-33
Identities = 94/111 (85%), Gaps = 0/111 (0%)
Strand=Plus/Plus

```

Query 16  TGCACCACTGCAAGAAGAGCTGCTGCTCATGCTGCCCATCAGGCTGCAGCAAGTGTGCC 75
          |||
Sbjct 73  TGTACTACCTGCAAGAAGAGTTGTTGTTCTTGCTGCCCATCTGGTTGCAGCAAGTGTGCC 132

Query 76  TCTGGCTGCGTGTGCAAAGGGAAGAAATGTGACACCAGCTGCTGTCAAGTGA 126
          |||
Sbjct 133 TCTGGCTGCGTGTGCAAAGGCAATTCCTGTGGCTCCAGCTGCTGTCAATGA 183

Score = 25.8 bits (55), Expect = 4e-05, Method: Compositional matrix adjust.
Identities = 13/17 (77%), Positives = 14/17 (83%), Gaps = 0/17 (0%)

Query 25  ASGCVCKGKKCDTSCCQ 41
          ASGCVCKG C +SCCQ
Sbjct 44  ASGCVCKGNSCGSSCCQ 60

```

B. Score = 652 bits (722), Expect = 0.0
Identities = 393/414 (95%), Gaps = 0/414 (0%)
Strand=Plus/Plus

```

Query 1  ATCCCCGACTGCTCGGCTGAGAAAGCACTGATGTACCAGCGCATGTTTGAGGGTCTCACG 60
          |||
Sbjct 265 ATCCCCGACTGCTCGGCTGAGAAAGCACTGATGTACCAGCGCATGTTTGAGGGTCTCACG 324

Query 61  CTCACCCAGAAAATGGCTGATGTTATCTACTACAAGTCCCTGAGGGAGAGAGA 120
          |||
Sbjct 325 CTCACCCAGAAAATGGCTGATGTTATCTACTACAAGTCCCTGAGGGAGAGAGA 384

Query 121 CACGACTCTGCTGTGAAGAGAAACAGAGAGGCCCTGAGTGTGAGGTCAAACGTGGGAG 180
          |||
Sbjct 385 CACGACTCTGCTGTGAAGAGAAACAGAGAGCGTCTGAGTGTGAGGTCAAGCTGTGGGAG 444

Query 181 GGATACCTGCAGAAGGCATCAGGCCCTTCTTGGCAGGAAAGAACTTTTCTTGGCTGAT 240
          |||
Sbjct 445 GGATACCTGCAGAAGGCATCAGGCTCTTCTTGGCAGGAAAGAACTTTTCTTGGCTGAT 504

Query 241 GTGATCGTTTATCCATCCATCGCTTATATCTTCCACTTTGGGTTATGTGAAGAGCGTTAC 300
          |||
Sbjct 505 GTGACGGTTTATCCATCTATCGCTTATCTCTTCCATTTTGGGTTGTGTGAAGAGCGTTAC 564

Query 301 CCTAAACTGGCAGCTTACTATAACGCCAATAAGGAGAGACCCAGCATCAAAGCCACATGG 360
          |||
Sbjct 565 CCTAAACTGGCAGCTTACTATAACTCCAATAAGGACAGACCCAGCATCAAAGCCACATGG 624

Query 361 CCTCCTTCTTGGCTGGAGAGCTCACAGGGACAAGACCAACTGAAAGACATTTGA 414
          |||
Sbjct 625 CCTCCTACCTGGCTGGAGAACCACAGGGACAAGACCAACTGAAAGACATTTGA 678

Score = 267 bits (683), Expect = 6e-77, Method: Compositional matrix adjust.
Identities = 124/136 (92%), Positives = 130/136 (96%), Gaps = 0/136 (0%)

Query 1  IPDCSAEKALMYQRMFEGTLTLQKMDVYYNWKVPEGERHDSAVKRNREALSAEVKLWE 60
          |||
Sbjct 89  IPDCSAEKALMYQRMFEGTLTLQKMDVYYNWKVPEGERHDSAVKRNRLVLSAEVKLWE 148

Query 61  GYLQKASGFFLAGKNFSLADVYPYIAYIFHFGCLCEERYPKLAAYYNANKERPSIKATW 120
          |||
Sbjct 149 GYLQKASGSFFAGKNFSLADVTYPYIAYLFFHFGCLCEERYPKLAAYYNSNKDRPSIKATW 208

Query 121 PPSWLESSQGQDQLKD 136
          PP+WLE+ QGQDQLKD
Sbjct 209 PPTWLENPQGQDQLKD 224

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Figure 3. The nucleotide and protein alignments (A) of the longear sunfish metallothionein-2 (query) and zebrafish (*Danio rerio*) metallothionein-2 (subject) gene and protein, and (B) the longear sunfish (*Lepomis megalotis*) glutathione-S-transferase (query) and largemouth bass (*Micropterus salmoides*) glutathione-S-transferase gene and protein.

Table 1. Partial sequences obtained for longear sunfish (*Lepomis megalotis*).

MT:TGCGCAAAGTGTGCTCCTGCACCACCTGCAAGAAGAGCTGCTGCTCATGCTGCCCATCAGGCTGCAGCAAGTGTGCCTCTGGCTGCGTGTGCAAAGGGAAGAAATGTGACACCAGCTGCTGTCAGTGA

GST:ATCCCCGACTGCTCGGCTGAGAAAGCACTGATGTACCAGCGCATGTTTGAGGGTCTCACGCTCACCCAGAAAATGGCTGATGTTATCTACTACAAGTCCCTGAGGGAGAGAGACACGACTCTGCTGTGAAGAGAAACAGAGAGGCCCTGAGTGCTGAGGTCAAAGTGTGGGAGGATACTGTCAGAAAGGCATCAGGCCCTTTCTGGCAGGAAAGAACTTTTCTTGGCTGATGTGATCGTTTATCCATCCATCGCTTATATCTTCCACTTTGGGTTATGTGAAGAGCGTTACCCTAAACCTGGCAGCTTACTATAACGCCAATAAGGAGAGACCCAGCATCAAAGCCACATGGCCTCCTTCTGGCTGGAGAGCTCACAGGGACAAGACCAACTGAAAGACATTTGA

Table 2. Partial sequences obtained for *Lepomis cyanellus* (green sunfish).

MT:TGCGCAAAGTGTGCTCCTGCACCACCTGCAAGAAGAGCTGCTGCTCATGCTGCCCATCAGGCTGCAGCAAGTGTGCCTCTGGCTGCGTGTGCAAAGGGAAGAAATGTGACACCAGCTGCTGTCAGTGA

GST:ATCCCCGACTGCTCGGCTGAGAAAGCACTGATGTACCAGCGCATGTTTGAGGGTCTCACGCTCACCCAGAAAATGGCTGATGTTATCTACTACAAGTCCCTGAGGGAGAGAGACACGACTCTGCTGTGAAGAGAAACAGAGAGGCCCTGAGTGCTGAGGTCAAAGTGTGGGAGGATACTGTCAGAAAGGCATCAGGCCCTTTCTGGCAGGAAAGAACTTTTCTTGGCTGATGTGATCGTTTATCCATCCATCGCTTATATCTTCCACTTTGGGTTATGTGAAGAGCGTTACCCTAAACCTGGCAGCTTACTATAACGCCAATAAGGAGAGACCCAGCATCAAAGCCACATGGCCTCCTTCTGGCTGGAGAGCTCACAGGGACAAGACCAACTGAAAGACATTTGA

CYP1A:GACACCTGTGTCTTCATCAATCAGTGGCAAATCAACCATGATCCGGAGTTGTGGAAAATGATCCGTCTTCCTTCAACCCAGATCGCTTCCTGAGTGCTGATGGCACTGAGCTCAACAAGCTGGAGGGGAGAAAGGTTACAACCTTTTGGCTTGGGAAAGCGGCGCTGCATCGGCGAGGTCATTGCACGAAATGAAGTCTACCTCTTCTTGGCAATCATGGTCCAGAAGCTGCACTTCAGCGCAATTCCTGGAGAGCCGCTGGACATGACCCAGAATACGGTCTCACAATGAAGCACAAACGCTGCAACCTGAGAGCCACGATGCGAGCGAGGGATCAGCAGTGAATGTATGAATGTGTTATTTCAATGTACAATGTTTGACTCAACAGGTGGTATGAGTTAACTCATAAAGCTGGATGGGTTATAGTCAAGTTAAGGGTTAGTGAAAGAAGCATCTCTCTCAGAATGTAAGGCACTGGATCCCGAAATGGATCCACAGAGCTACTGGCATTAAAGCAAATATGTGGATTTTGCTTGCTGCAGATTGTCAGAGATGCTTGGTTCTTATGAACCTATCTCTGTCATGTTTTGGTTTCCCAAGAGATATTCCTGCACATGACTGGTGCTACTCAGTGAAGTATAAGGATATACTTGGTTCTCCTGTGTTGGGTTGTGTAGTAACTGTTCCCTTAAGAAGTTGTAAAACACACAGAGACTATGTTTTGTTTGCTTTGTTCAACTCTGGGACAAACAGTATGCTTTACAGGATAGGCATTACTATTTAGACCACTGCAAACAATTTTTCTCTTCTGTGACACA

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Quantifying benthic macroinvertebrate communities and habitat in a recently restored stream in eastern Kentucky

Basic Information

Title:	Quantifying benthic macroinvertebrate communities and habitat in a recently restored stream in eastern Kentucky
Project Number:	2012KY198B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 5th
Research Category:	Biological Sciences
Focus Category:	Ecology, Surface Water, Methods
Descriptors:	floodplain, hydrologic function, channelization
Principal Investigators:	Amy Braccia

Publications

1. Revetta, Nicholas, Amy Braccia, Art Parola, Clayton Mastin, 2013, Quantifying Benthic Macroinvertebrate Community Structure and Biomass in a Recently Restored Stream in Eastern Kentucky, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p. 47-48.
2. Revetta, Nicholas, Amy Braccia, Art Parola, and Clayton Mastin, 2012, Quantifying Benthic Macroinvertebrate Community Structure, Biomass, and Habitat in a Recently Restored Stream in Eastern Kentucky, Poster presented at the Kentucky Academy of Science Annual Meeting, Richmond, KY.

Quantifying Benthic Macroinvertebrate Communities and Habitat in a Recently Restored Stream in Eastern Kentucky

Problem and Research Objectives

Slabcamp Creek is a first order tributary of the Licking River watershed and drains approximately 1 mi² in the Daniel Boone National Forest (Rowan Co., KY). Like many streams in Kentucky, including those that represent the headwater reference condition in Kentucky's statewide bioassessment program, Slabcamp Creek was straightened and moved to the base of a mountain in order to support farming in the valley many years ago. As a result of channelization and historical land use, sections of Slabcamp Creek lost connections to groundwater and its natural floodplain, which resulted in altered hydrology and habitat. The channel eroded to bedrock, benthic habitat was unstable during storm flow, and sections of the channel dried during late summer/early fall.

The USDA Forest Service decided to restore the hydrologic and habitat function in approximately 1 mi of Slabcamp Creek. The restoration design involved removing legacy sediment from the valley to expose gravels that formed the original stream riffles, the channel was relocated to its original aquifer and reconnected to its floodplain in the center of the valley, and wood was placed in the new channel to control gradients. The restoration of Slabcamp Creek was completed by the Stream Institute, University of Louisville, in October 2011. Our original project objective was to quantify and compare the benthic macroinvertebrate communities and habitat in Slabcamp Creek and a pre-restoration reference site during the first post-restoration year. In addition to the original objective, we estimated benthic invertebrate standing stock biomass in Slabcamp Creek and the pre-restoration reference site.

Methodology

Reference site selection. Because there was not an opportunity to perform pre-restoration collections, we worked with the USDA Forest Service and Dr. Parola (Stream Institute, University of Louisville) to select White Pine Branch as our pre-restoration reference site. White Pine Branch was chosen as the pre-restoration reference site because: (1) it is located within the same bioregion, (2) has a similar watershed size and land use, and (3) has the same historical disturbance (channelization) as Slabcamp Creek. Dr. Parola began hydrologic monitoring at White Pine Branch during late summer 2012 in support of our study.

Seasonal, quantitative benthic collections: We sampled macroinvertebrates seasonally (fall 2011, winter 2012, spring 2012, summer 2012) throughout the first post-restoration year in a 150 m representative reach in each stream. We used a systematic sampling design to select sampling locations from the dominant, wetted-channel habitats. A modified Hess sampler (0.086 m², 250 μ m mesh) was used to collect 5 replicate benthic samples from riffles and pools. At each sample location we collected macroinvertebrates, their food resources (fine and coarse benthic organic matter), and physical habitat (depth, flow and visual estimates of substrate composition). Our sampling design resulted in 10 benthic samples (5 from riffles and 5 from pools) from each stream in each season. During summer, we were able to collect only 5 replicates from pools in Slabcamp because flow in riffles was too low for the Hess sampler and the channel at White Pine

Branch was dry. In total, we collected 65 benthic samples during the first post-restoration year. The pool samples ($n=5$) collected from Slabcamp Creek during summer were not included in our analysis.

Laboratory methods: All benthic samples were processed by sorting macroinvertebrates from organic material using a dissecting microscope. Macroinvertebrates were identified to the lowest practical taxonomic level (mostly genus, except Chironomidae), enumerated, and measured to the nearest 0.5 mm in order to estimate community biomass from published length-weight regressions. Coarse and fine benthic organic matter was retained, dried, weighed and combusted in order to report standing crops of organic matter.

Data analysis: Data analysis consisted of several steps. First, we compared macroinvertebrate densities and biomass between sites. Second, we will compare community composition between sites with multivariate ordinations and taxonomic-based (e.g. %EPT, Simpson's Diversity) and trait-based (e.g., %univoltine, #clinger taxa) metrics. Finally, our measures of food resources and physical habitat will be related to macroinvertebrate community data.

Principle Findings and Significance

Thus far, our analysis shows that Slabcamp Creek had significantly greater macroinvertebrate abundance ($t_{58} = 2.79$, $p = 0.007$) and biomass ($t_{58} = 4.71$, $p < 0.001$) for all benthic samples combined (seasons and habitats). Further, there were habitat-specific differences between streams; no difference in densities or biomass was detected between pools and riffles in Slabcamp, but riffles in White Pine had significantly less density and biomass than pools (Fig 1).

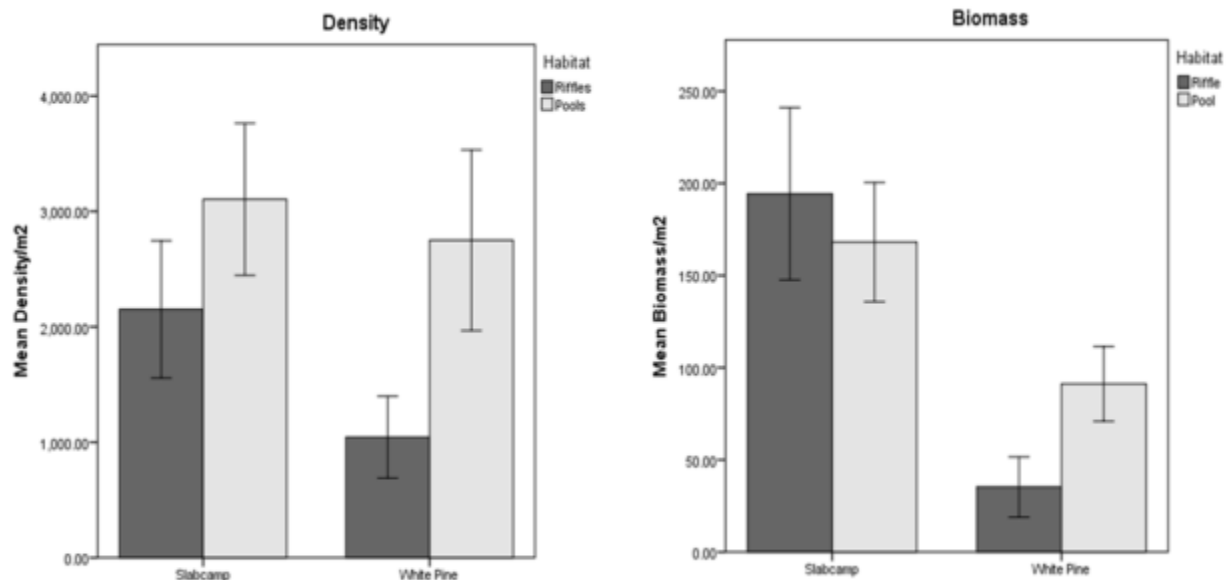


Fig. 1. Mean (± 1 SE) macroinvertebrate densities and biomass from riffles ($n = 15$) and pools ($n=15$) in Slabcamp and White Pine Branch during the first, post-restoration year. Two-way ANOVA for densities: Site $F_{(1, 59)} = 8.407$, $p = 0.005$; Habitat $F_{(1, 59)} = 5.193$, $p = 0.027$; Site \times Habitat $F_{(1, 59)} = 0.207$, $p = 0.651$. Two-way ANOVA for biomass: Site $F_{(1, 59)} = 20.812$, $p < 0.001$; Habitat $F_{(1, 59)} = 9.794$, $p = 0.003$; Site \times Habitat $F_{(1, 59)} = 2.097$, $p = 0.153$.

When biomass estimates were weighted to total available riffle habitat, which was determined from seasonal reach-scale habitat measures, Slabcamp Creek had nearly 9 times more benthic invertebrate biomass than the reference site in spring and summer (Table 1).

Table 1. Invertebrate standing stock biomass (g AFDM) from riffles. Values were determined by multiplying seasonal estimates (mg AFDM/m²) by total wetted riffle habitat in each stream

	Fall	Winter	Spring
Slabcamp Creek	85.5	47.9	57.1
White Pine Branch	33.2	5.5	6.3

Preliminary analysis of macroinvertebrate community structure from riffles show that Slabcamp Creek had higher total taxa richness (Slabcamp creek = 52 taxa, White Pine Branch = 45 taxa) and a higher proportion of EPT taxa (Slabcamp Creek %EPT = 54, White Pine Branch %EPT = 47). Hydrologic monitoring indicated the restoration practice improved surface flow throughout the critical flow period (late summer/early fall) (Fig. 2).

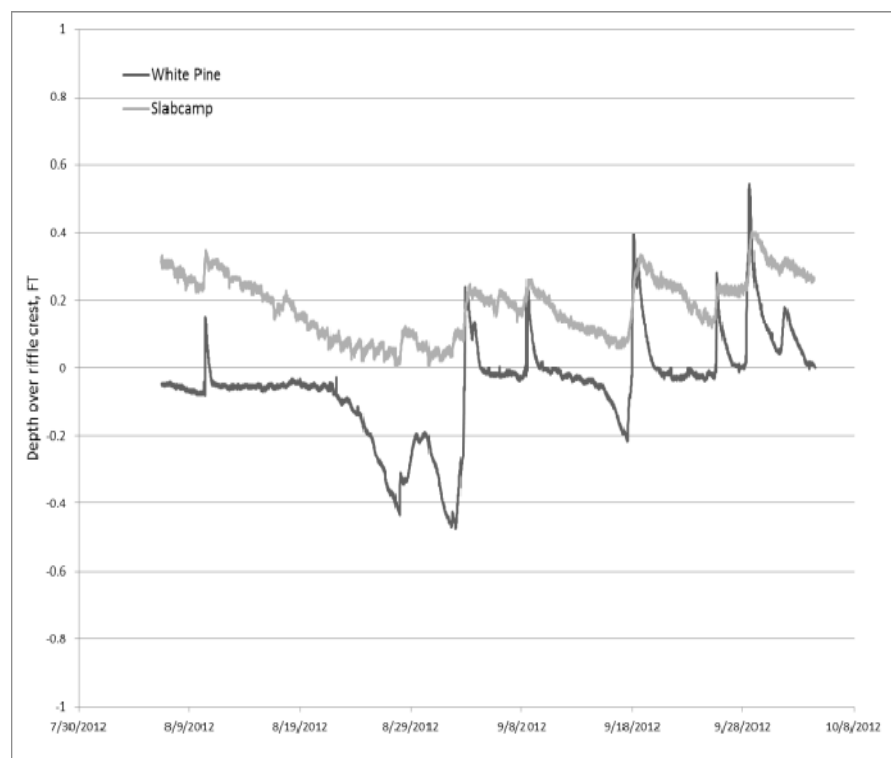


Fig. 3 A) Depth of water over riffle crests during late summer 2012. Negative values imply no surface flow in riffles and that channel bed had begun to dry; if surface water was present, it was restricted to pools. Positive values indicate surface flow throughout the channel (in riffles and pools).

Preliminary results from the first post-restoration year show a positive improvement in macroinvertebrate community biomass relative to our reference site. Our leading hypothesis is

that restored hydrologic functions in Slabcamp Creek resulted in stable benthic habitat which is reflected in invertebrate biomass. Our trait-based community analysis might provide further support for this hypothesis, but this needs to be confirmed by expanding our methods to other restoration and reference sites.

Development of wetland assessment methods for Kentucky

Basic Information

Title:	Development of wetland assessment methods for Kentucky
Project Number:	2012KY199B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 6th
Research Category:	Biological Sciences
Focus Category:	Wetlands, Ecology, Surface Water
Descriptors:	vegetation, rapid assessment methods, landscape development intensity index
Principal Investigators:	David Brown

Publications

1. Morris, Tanner M., JohnRyan A. Polascik, David R. Brown, 2013, Developing a Vegetation-Based Index of Biotic Integrity for Assessing the Ecological Condition of Wetlands in Kentucky, in Proceedings of the Kentucky Annual Water Resources Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p. 39 (also presented at the 2012 Kentucky Academy of Science meeting in Richmond, KY and the 2013 Association of Southeastern Biologist meeting in Charleston, WV)
2. Polascik, JohnRyan A., David R. Brown, and Tanner M. Morris, 2013, Using vegetation and Landscape Analysis to validate a wetland rapid assessment method for Kentucky's Forested Riparian Wetlands, in Proceedings of the Kentucky Annual Water Resources Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY p. 41-42 (also presented at the 2012 Kentucky Academy of Science meeting in Richmond, KY and the 2013 Association of Southeastern Biologist meeting in Charelston, WV)

Development of Wetland Assessment Methods for Kentucky

Problem and Research Objectives

Over the last two centuries, Kentucky has lost more than 80% of its historic wetlands (Dahl and Johnson, 1991). Land development, agriculture, mining, and other human disturbances have contributed to this decline through dredging, draining, filling, leveling, and flooding of wetlands. Wetlands provide many benefits for humans and the environment alike, including flood mitigation, sediment load reduction, nutrient removal and retention, carbon sequestration, food production, biodiversity, and critical habitat for many species of organisms. In addition, it has been argued that wetlands are classified as some of the most valuable habitat annually per hectare worldwide (Costanza et al., 1997). Although a "no net loss" policy of total wetland acreage has been put into action, there has been less attention given to the ecological integrity (quality) of wetlands despite the fact that ecological integrity is correlated with the value of functions and services that they can provide (Mack, 2007). Ecological integrity can only be measured through quantitative and qualitative assessment; however, Kentucky currently has no statewide methods to measure the ecological integrity of wetlands. Recently, a cooperative project between Eastern Kentucky University, the Kentucky Division of Water, and an interagency technical committee has begun the process to develop a wetland rapid assessment method for the state. When implemented, this assessment method will provide a regulatory tool for state and federal agencies to better manage wetland resources in Kentucky, with the intention that it will create a higher permitting threshold or increase mitigation ratios for development of high quality wetlands.

Biological integrity is the ability of an ecosystem to support a relatively high quality and functional biological community (Karr and Dudley, 1981). One of the objectives set forth through the Clean Water Act of 1977 was to "restore and maintain the physical, chemical and biological integrity of the Nation's waters" (33 U.S.C. Section 1251). Wetlands are a type of "water" of the United States and jurisdictional wetlands of Kentucky fall under this category.

Ecological integrity of a wetland has been correlated with human disturbance (U.S. EPA 2002a). The degree and impact of disturbance can be measured through qualitative and quantitative assessment methods. Historically, wetland assessment has been organized with a 3-level framework. Level 1 methods are broad landscape-scale assessments typically based on remote-sensing data. Level 2 methods are on-the-ground rapid assessments typically requiring less than 1 day in the field. Level 3 methods are intensive assessments using biotic indicators or physiochemical analysis as endpoints (Fennessy et al., 2007). Each of these three levels can be used in the validation of another level. This study focused on the development and validation of Level 2 and Level 3 assessments for Kentucky's wetlands.

In response to the Clean Water Act's goal of restoring the biological integrity of waters of the United States, a number of indices of biotic integrity (IBI) have been developed using various taxonomic groups (Mack, 2007a). For wetlands, the taxonomic group best suited for wetland assessment is vascular plants. Since IBIs are considered Level 3 assessments, they can be used to validate Level 2 assessments.

The objectives of this project were (1) to develop a vegetation-based IBI (level 3) method for Kentucky based on existing methods previously developed in Ohio, and (2) validate a rapid assessment method for Kentucky recently developed by a multi-agency technical committee using the vegetation-based IBI and a Level 1 assessment. Ultimately, the goal is to develop methods that will be applicable statewide in all types of wetlands, but since this project is in the relatively early stages of development, the objective for this project was to develop and validate methods for riverine wetlands in Eastern and Central Kentucky.

Methodology

A total of 43 wetlands were sampled during the summers of 2011 and 2012. Sites were selected within the Green (n = 17), Upper Cumberland (n = 19) and Kentucky (n = 7) River basins of Western, South-Central and Central Kentucky, respectively. The National Wetland Inventory (NWI) database was used to select and verify wetland existence and classification as either forested (n = 28) or emergent (n = 15) and all wetlands had a riverine hydrology.

At each site, a Kentucky Wetland Rapid Assessment Method (KYWRAM) was conducted. The protocol used for this followed the latest draft of the KYWRAM. The KYWRAM was conducted by multiple individual “raters” and was completed within a few hours upon surveying the site. All raters conducting a KYWRAM received similar training prior to the field season in an attempt to avoid bias results. Scores were then averaged across raters. The KYWRAM was completed on the same day as the vegetation survey. The KYWRAM contains 6 metrics of disturbance and scored an individual wetland between 0 and 100.

At each site, intensive vegetation data was collected using the Ohio Vegetation Index of Biotic Integrity protocol (Mack, 2007b), but modified for Kentucky’s vegetation. The vegetation survey of each wetland was conducted using a series of 10 continuous plots or “modules” in a 2x5 arrangement numbered 1 through 10 counterclockwise (Peet et al., 1998). Each module had a dimension of 10m² (0.01ha). Of the 10 modules, four (modules 2, 3, 8 and 9) were sampled intensively and six (modules 1, 4, 5, 6, 7 and 10) were used for residual surveys. Intensive modules were surveyed for plant species at four scales of quadrats: 0.01m², 0.1m², 1m² and 10m². Surveys in the smallest three quadrat sizes were conducted at two opposite corners of a module. All plants that fell within the module were identified to the species level, and a cover class was assigned for each species in the module. Any specimen that could not be properly identified in the field was collected and pressed for later identification. Voucher specimens for each wetland were collected and used for reference within each site. For forested wetlands, trees were identified to species in every module and the diameter at breast height (dbh) was recorded for each individual tree. For emergent wetlands, a 1 m² sample of above ground biomass was collected from each of the four intensive modules. The biomass samples were dried for 24 hours and weighed to the nearest 0.1 g.

For each site, a Landscape Development Intensity Index (LDI) was conducted. LDI analysis was done using a combination of Geographic Information Systems (ArcMap10) and ground-truthing. The LDI was calculated as follows,

$$LDI_{total} = \sum \%LU_i \cdot LDI_i$$

where, LDI_{total} is the LDI ranking for landscape unit, $\%LU_i$ is the percent of the total area of influence in land use i , and LDI_i is the landscape development intensity coefficient for land use i (Brown and Vivas, 2005). LDI scores were calculated on a scale of 1 through 10, where 10 defines a completely disturbed area and 1 is a high-quality reference habitat. The primary GIS layer for this analysis was the National Land Cover Dataset (NLCD) from 2006 with a 30 m² pixel resolution. Each pixel has a designated land use type that was assigned an LDI coefficient to be used in the calculation. We calculated the LDI_{total} within a 1000m buffer around the point of the VIBI survey. Since this study was conducted in an ecoregion similar to Ohio, we followed the LDI coefficients of Mack (2007a).

Principal Findings and Significance

We used linear regression to compare all three methods of wetland assessment. The results show similar trends for both forested and emergent wetlands and follow predictions in that the KYWRAM and VIBI are positively correlated with one another and both are negatively correlated with the LDI. The KYWRAM showed a non-significant, positive relationship with the VIBI in the forested sites ($R^2 = 0.063$, $F_{1, 26} = 1.752$, $p = 0.200$) and no relationship in the emergent sites ($R^2 = 0.003$, $F_{1, 13} = 0.036$, $p = 0.85$) (Figure 1a, 1b). The KYWRAM showed a significant, negative relationship with the LDI in the forested sites ($R^2 = 0.252$, $F_{1, 26} = 8.777$, $p = 0.006$) and emergent sites ($R^2 = 0.355$, $F_{1, 13} = 7.153$, $p = 0.019$), respectively (Figure 1c, 1d). The VIBI showed a marginally significant, negative relationship with the LDI in the forested sites ($R^2 = 0.131$, $F_{1, 26} = 3.936$, $p = 0.058$) and the emergent sites ($R^2 = 0.189$, $F_{1, 13} = 3.036$, $p = 0.110$), respectively (Figure 1e, 1f). The results suggest landscape disturbance was an effective predictor of wetland condition and integrity as assessed by the KYWRAM and VIBI. However, there was no strong relationship when the KYWRAM was compared to the VIBI. Since both of these methods are in the developmental stage, a more detailed analysis of metric performance is necessary. Additionally, the results suggest that our experimental design did not adequately represent both low quality and high quality wetlands. Notice in Figure 1 panels a-d that relatively few wetlands were sampled with KYWRAM scores below 30 and above 80. This is not totally surprising given our randomized sampling design. Future work in the upcoming year will aim to capture a more complete disturbance gradient by targeting more low and high quality wetlands. The KYWRAM and VIBI development will also focus on individual metric performance. Since both of these methods were adopted from Ohio, we will select and revise metrics that best represent Kentucky's wetlands.

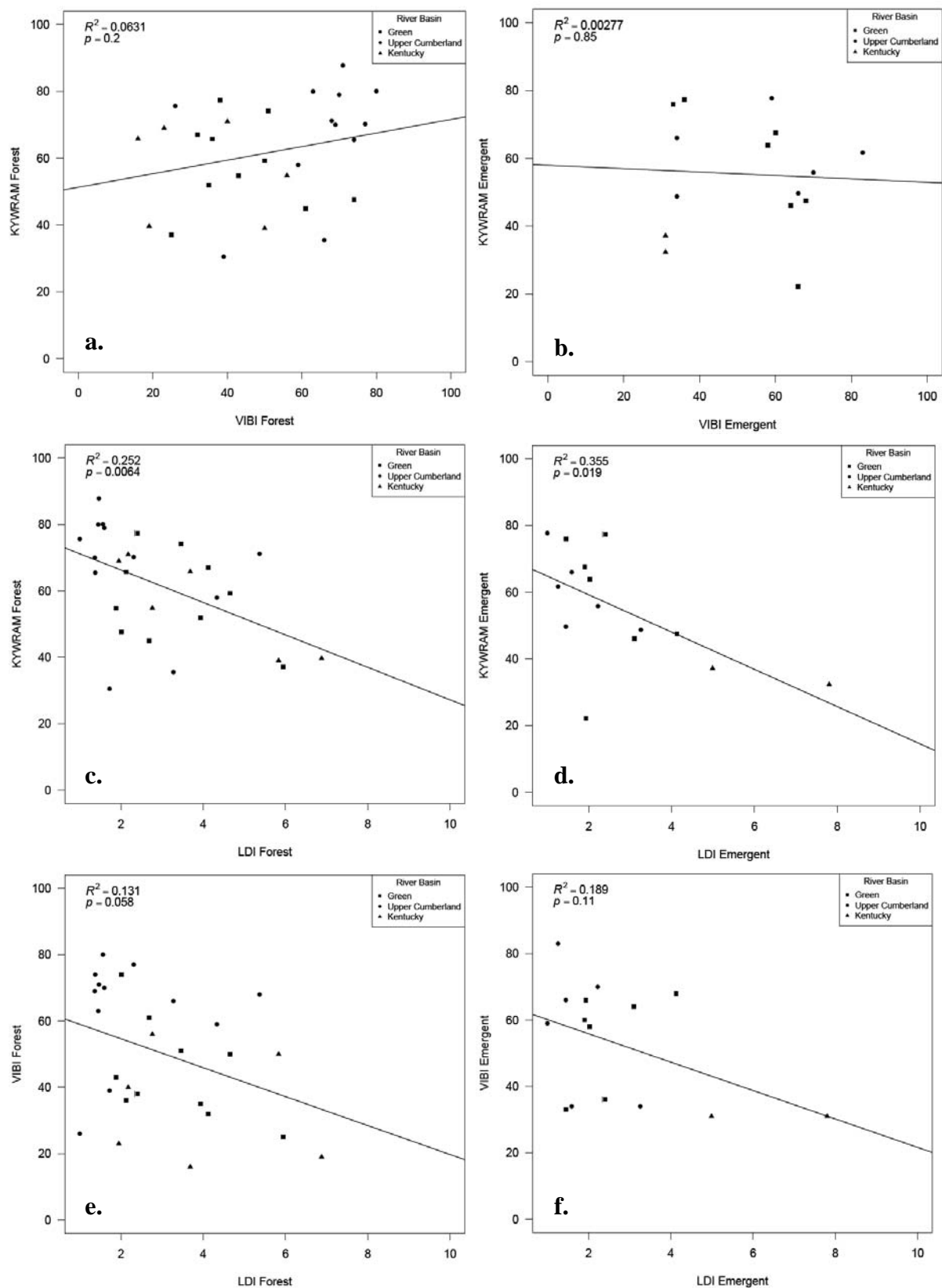


Figure 1. Linear relationships between (a) KYWRAM and VIBI, (b) KYWRAM and LDI, (c) VIBI and LDI for forested wetlands and (d) KYWRAM and VIBI, (e) KYWRAM and LDI, and (f) VIBI and LDI for emergent wetlands.

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Hydrologic characterization of a tree- and shrub-vegetated rain garden

Basic Information

Title:	Hydrologic characterization of a tree- and shrub-vegetated rain garden
Project Number:	2012KY200B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 6th
Research Category:	Climate and Hydrologic Processes
Focus Category:	Surface Water, Hydrology, Management and Planning
Descriptors:	stormwater, LID, infiltration
Principal Investigators:	Carmen Agouridis

Publications

1. McMaine, John and Carmen Agouridis, 2013, Hydrologic Characterization of a Tree- and Shrub-Vegetated Rain Garden in Central Kentucky, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p. 23-24
2. McMaine, John and Carmen Agouridis, 2013, Evaluating the Hydrologic Effectiveness of a Rain Garden in Central Kentucky, 2013 Student Water Conference, Oklahoma State University, Stillwater, OK, April 4-5, 2013.
3. Agouridis, Carmen and John McMaine, 2013, AEN-118 Managing Stormwater Using Low Impact Development (LID) Techniques, University of Kentucky Cooperative Extension Service Publication, University of Kentucky, Lexington, KY.

Hydrologic Characterization of a Tree- and Shrub-Vegetated Rain Garden in Central Kentucky

Problem and Research Objectives

Urbanization increases the volume of stormwater generated through the addition of impervious surfaces such as parking lots, rooftops, and paved surfaces (Agouridis et al., 2011). Such increases in imperviousness have led to reduced levels of infiltration and subsequently increase in peak flows and total runoff volumes. These hydrologic impacts contribute to reductions in groundwater recharge, as runoff is quickly directed to the storm sewer system instead of being allowed to infiltrate. Traditional stormwater management practices have focused on capturing, detaining, and releasing stormwater flows through structures such as detention ponds (Selbig and Balster, 2010; Plueger, 2011). Under the new Environmental Protection Agency (EPA) performance guidance, the focus is shifting to management strategies that capture and retain via infiltration or rainwater harvesting (Plueger, 2011). Rain gardens, which are structures that use a conditioned planting bed and landscaping in a shallow depression, are one means of promoting infiltration of runoff from impervious surfaces (Selbig and Balster, 2010; Agouridis et al., 2011). While studies indicate that rain gardens are an effective way of infiltrating runoff (Dietz and Clausen, 2005; Dietz and Clausen, 2006), as noted by Selbig and Balster (2010), these studies are not truly representative, since water from the rain garden was not allowed to infiltrate into the groundwater but was captured by an underground impermeable layer for purposes of volumetric water-mass balance computations. Additionally, none of these studies were conducted in Kentucky. As such, the Lexington-Fayette Urban County Government (LFUCG) expressed a keen interest in understanding the ability of rain gardens to infiltrate stormwater runoff in Fayette County, Kentucky.

The specific research objectives of the project included:

- Characterize hydrologic performance of the rain garden
 - Perform water balance
 - Quantify infiltration rates
 - Quantify evapotranspiration rates
- Make design recommendations using performance observations
- Compare performance with performances found in similar rain garden studies

Methodology

In September 2011, Coca-Cola Enterprises constructed a second rain garden at their Leestown Road bottling facility (Figure 1). This rain garden is approximately 10,000 ft² and receives runoff from the warehouse roof and parking lot via a 12-inch diameter inlet pipe and a 10-inch diameter inlet pipe. Planted in October 2011, the rain garden is dominated by native trees and shrubs. A limestone walking path, in the shape of the iconic Coca Cola wave, traverses the rain garden. Additionally, a 12,500 gallon infiltration chamber is located at the edge of the rain garden. The infiltration chamber was installed because the performance of rain

gardens with soils found in Fayette County (silt loams) is not well known. When water reaches a defined elevation, a 12-inch overflow pipe carries water into the infiltration chamber. Once the infiltration chamber fills, the rain garden depression can continue to fill before overtopping. The construction of the rain garden also included loosening and amending the soil. The top 4 feet of soil was amended with a mixture of sand, compost, and mulch. From 4 feet to 8 feet below the ground surface, the soil was loosened using a backhoe. This rain garden was constructed without a liner or an underdrain system, so it is more representative of a typical rain garden design.

Hydrologic characterization at the newly built Cocal Cola rain garden follows the procedures outlined by Selbig and Balster (2010). A tipping bucket rain gage was installed at the project site to record precipitation data (e.g. depth and intensity). Due to equipment failure and user error, an alternate source for precipitation data was needed. Precipitation data from USGS gauging station 03289200 (Town Branch at Yarnallton Road) was used. The gauging station is located 3.2 miles from the project site and records precipitation every 5 minutes. Estimates of evapotranspiration will be computed to characterize the volume of water lost to the atmosphere. Weather data from the University of Kentucky's Spindletop Farm (within 5 miles of the project site) will be used to compute reference evapotranspiration rates using the Penman-Monteith equation. Stormwater runoff influent was continuously monitored using two Teledyne-ISCO 4200 series flow meters (one per inlet pipe) (Figure 2). Effluent was monitored using an In-situ Level Troll 500 (5 psig) pressure transducer, which was installed in the overflow pipe. A detailed survey of the rain garden was conducted, and a stage-volume relationship developed to assess effluent volume and stored volume. Following the cessation of rainfall, infiltration rates are estimated based on the rate of falling head as outlined by Selbig and Balster (2010). The volumetric soil-moisture profile has been measured using vertically oriented soil moisture sensors installed at 20 cm increments to a depth of about four feet (Figure 3). A well was installed at a depth of approximately 3.5 ft and an In-situ Level Troll 500 (5 psig) pressure transducer was installed at the bottom of the well to monitor water table level. Hydrologic performance is being assessed for each rain event.

Principal Findings and Significance

Data collection was completed on May 1, 2013. Over the monitoring period of March 2012 through April 2013, approximately 50 storms occurred that produced a significant amount of runoff (Figure 4). As expected, most of the rain events were small and only a few large rain events occurred (Figure 5). Preliminary results indicate that for the storms analyzed, infiltration rates were on the order of 6 to 8 in hr⁻¹. It is visually apparent that there is a period of rapid infiltration followed by a lower "steady-state" infiltration rate (Figure 6). Infiltration rates were determined by observing when inflow stopped and then looking at the rate of change of the water table level until it reached an equilibrium point. These infiltration rates will be compared to the findings from the soil moisture sensor. The soil moisture sensor was used to not only determine the water table level, but also to illustrate how the wetting front moves through the soil column. Continued analysis will include graphing each precipitation event and determining infiltration rates for each. Also, weather data from Spindletop will be used to determine evapotranspiration rates. To complete the water balance, storage within the soil void space will be determined. Determining infiltration rates will allow stormwater manuals to incorporate

these infiltration rates into their design recommendations. Currently, rain gardens are designed to be able to hold the volume of the design storm above ground, and assume that little to no infiltration occurs. These preliminary results show that infiltration occurs immediately, and it significantly influences what happens to water that has entered the rain garden. Completion of a thesis by John McMaine including the complete results of the investigation is expected by September 1, 2013.

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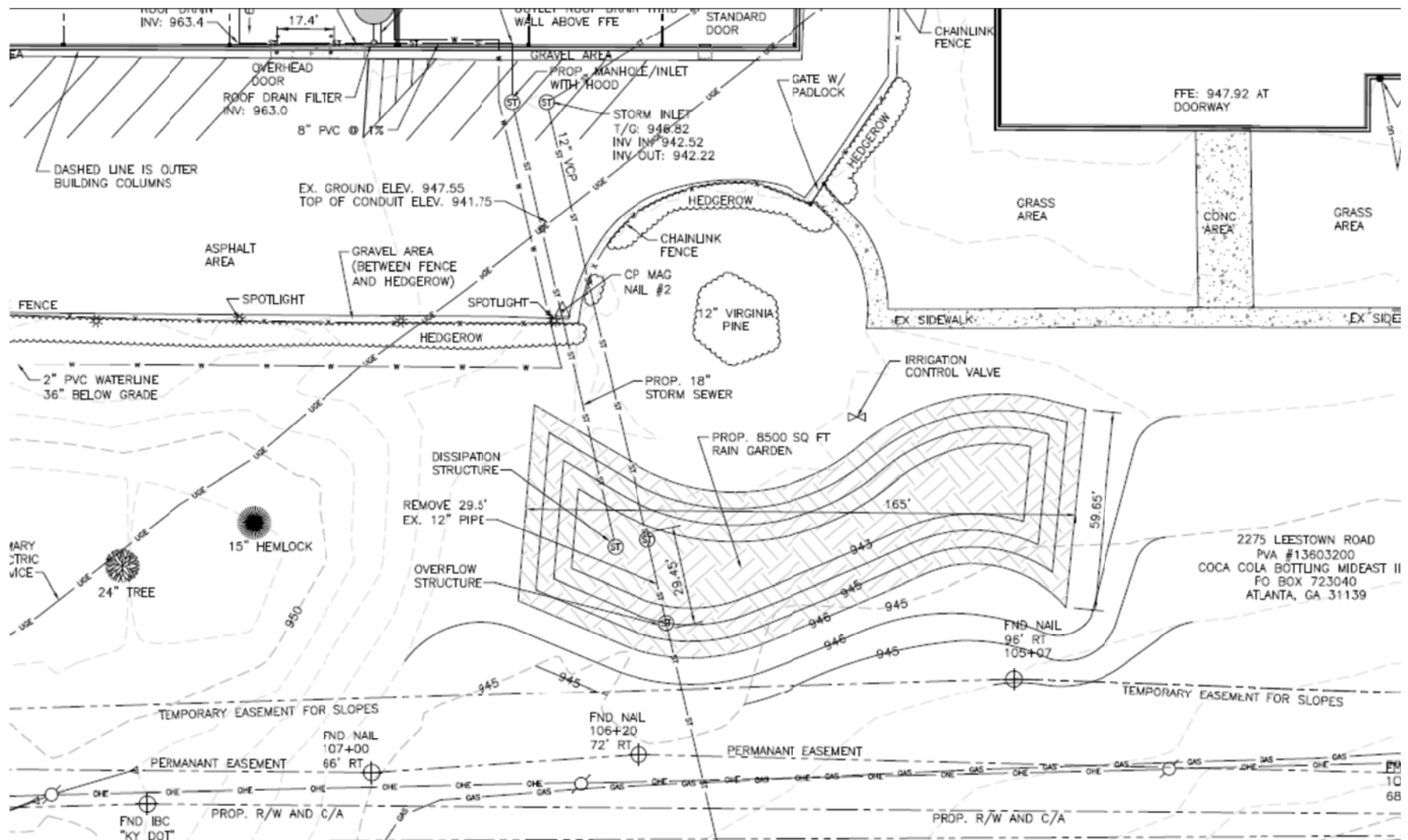


Figure 1: Coca Cola rain garden location and design.



Figure 2: Data acquisition types and locations.



Figure 3: Soil moisture sensor components and location.

Rainfall						Inflow					
Start Time (Date Time)	End Time (Date Time)	Duration (hr:min)	Duration (hr.decimal of hr)	Depth (in)	Avg Intensity (in/hr)	Start Time (Date Time)	End Time (Date Time)	Duration (hr:min)	Duration (hr.decimal of hr)	Total Volume (ft ³)	Peak Flow (ft ³)
1/13/13 5:30	1/13/13 20:55	15:25	15.42	2.20	0.14	1/13/2013 5:30	1/13/2013 23:20	17:50	17.83	11,755	0.79
9/25/12 10:20	9/25/12 13:25	3:05	3.08	2.08	0.67	9/25/2012 9:20	9/25/2012 13:50	4:30	4.50	3,972	0.83
12/9/12 20:05	12/10/12 14:10	18:05	18.08	1.76	0.10	12/9/2012 20:00	12/10/2012 15:10	19:10	19.17	6,119	0.83
7/14/12 14:00	7/14/12 17:30	3:30	3.50	1.37	0.39	7/14/2012 15:50	7/14/2012 17:40	1:50	1.83	1,794	1.41
5/12/12 20:10	5/13/12 19:10	23:00	23.00	1.30	0.06	5/12/2012 20:10	5/14/2012 6:00	9:50	33.83	9,162	0.61
12/20/12 7:05	12/20/12 17:15	10:10	10.17	1.21	0.12	12/20/2012 7:10	12/21/2012 12:20	5:10	29.17	6,289	0.46
7/23/12 13:30	7/23/12 13:40	0:10	0.17	1.10	6.60	Insignificant inflow					
9/2/12 16:10	9/3/12 10:35	18:25	18.42	1.00	0.05	9/2/2012 15:20	9/3/2012 8:40	17:20	17.33	7,646	1.01
9/28/12 6:40	9/28/12 15:30	8:50	8.83	0.93	0.11	9/28/2012 6:00	9/28/2012 18:30	12:30	12.50	4,216	1.14
11/12/12 5:35	11/12/12 14:35	9:00	9.00	0.84	0.09	11/12/2012 5:00	11/12/2012 15:40	10:40	10.67	7,017	1.20
3/11/13 7:15	3/11/13 16:05	8:50	8.83	0.84	0.10	3/11/2013 7:30	3/11/2013 18:30	11:00	11.00	5,201	0.54
5/31/12 21:10	6/1/12 2:55	5:45	5.75	0.82	0.14	5/31/2012 20:30	6/1/2012 2:40	6:10	6.17	2,900	1.11
3/8/12 9:50	3/8/12 19:00	9:10	9.17	0.72	0.08	3/8/2012 9:40	3/8/2012 20:50	11:10	11.17	4,819	0.36
4/21/12 2:00	4/21/12 9:25	7:25	7.42	0.67	0.09	4/21/2012 1:20	4/21/2012 9:50	8:30	8.50	3,129	0.28
12/7/12 6:25	12/7/12 22:30	16:05	16.08	0.67	0.04	12/7/2012 6:40	12/7/2012 20:50	14:10	14.17	2,365	0.42
6/11/12 4:20	6/11/12 11:25	7:05	7.08	0.66	0.09	6/11/2012 1:30	6/11/2012 11:10	9:40	9.67	3,469	0.63
5/7/12 19:15	5/8/12 4:20	9:05	9.08	0.63	0.07	5/7/2012 18:10	5/8/2012 3:50	9:40	9.67	4,208	2.03
9/26/12 3:25	9/26/12 7:20	3:55	3.92	0.59	0.15	9/26/2012 2:40	9/26/2012 7:30	4:50	4.83	1,238	0.43
12/4/12 17:45	12/4/12 20:05	2:20	2.33	0.59	0.25	12/4/2012 17:50	12/4/2012 23:30	5:40	5.67	4,242	1.25
3/17/12 23:15	3/18/12 0:15	1:00	1.00	0.58	0.58	3/17/2012 22:10	3/18/2012 2:00	3:50	3.83	3,373	1.78
12/31/12 12:50	1/1/13 17:05	4:15	28.25	0.57	0.02	12/31/2012 13:40	1/1/2013 4:50	15:10	15.17	1,471	0.15
9/8/12 1:30	9/8/12 4:35	3:05	3.08	0.57	0.18	9/8/2012 0:50	9/8/2012 5:00	4:10	4.17	2,980	1.45
5/5/12 1:10	5/5/12 3:15	2:05	2.08	0.55	0.26	5/4/2012 23:50	5/5/2012 7:10	7:20	7.33	3,214	1.38
9/17/12 15:20	9/18/12 4:05	12:45	12.75	0.51	0.04	9/17/2012 12:20	9/18/2012 9:00	20:40	20.67	6,132	0.48
12/17/12 16:10	12/17/12 21:10	5:00	5.00	0.49	0.10	12/17/2012 16:10	12/17/2012 23:50	7:40	7.67	3,491	1.26
3/15/12 17:40	3/16/12 4:45	11:05	11.08	0.48	0.04	3/15/2012 16:10	3/16/2012 5:50	13:40	13.67	1,220	0.33
9/5/12 17:45	9/5/12 19:15	1:30	1.50	0.48	0.32	9/5/2012 17:00	9/5/2012 19:30	2:30	2.50	1,330	0.90
12/25/12 23:55	12/26/12 15:45	15:50	15.83	0.44	0.03	12/26/2012 0:00	12/26/2012 17:10	17:10	17.17	1,954	0.36
6/17/12 9:40	6/17/12 10:10	0:30	0.50	0.41	0.82	6/17/2012 8:50	6/17/2012 10:10	1:20	1.33	1,555	1.82
4/4/12 14:40	4/4/12 21:10	6:30	6.50	0.41	0.06	4/4/2012 13:20	4/4/2012 19:00	5:40	5.67	2,072	0.71
1/11/13 6:45	1/11/13 9:35	2:50	2.83	0.38	0.13	1/11/2013 6:40	1/11/2013 11:10	4:30	4.50	2,289	1.41
6/17/12 14:45	6/17/12 17:50	3:05	3.08	0.35	0.11	6/17/2012 14:50	6/17/2012 18:30	3:40	3.67	1,966	1.91
10/14/12 23:00	10/14/12 23:05	0:05	0.08	0.31	3.72	10/14/2012 22:00	10/14/2012 23:00	1:00	1.00	581	0.59
11/26/12 20:25	11/27/12 1:50	5:25	5.42	0.30	0.06	11/26/2012 20:10	11/27/2012 5:30	9:20	9.33	1,000	0.21
7/1/12 21:55	7/1/12 23:30	1:35	1.58	0.27	0.17	7/1/2012 18:40	7/2/2012 2:40	8:00	8.00	349	0.17
12/4/12 9:00	12/4/12 14:40	5:40	5.67	0.26	0.05	12/4/2012 9:00	12/4/2012 14:00	5:00	5.00	1,971	0.51
12/9/12 8:50	12/9/12 14:15	5:25	5.42	0.26	0.05	12/9/2012 9:00	12/9/2012 12:40	3:40	3.67	2,053	0.75
11/3/12 15:15	11/3/12 18:40	3:25	3.42	0.26	0.08	11/3/2012 12:20	11/3/2012 21:20	9:00	9.00	589	0.20
3/2/12 17:05	3/2/12 17:45	0:40	0.67	0.26	0.39	3/2/2012 17:00	3/2/2012 19:40	2:40	2.67	1,421	1.02
9/8/12 7:45	9/8/12 9:25	1:40	1.67	0.25	0.15	9/8/2012 6:30	9/8/2012 10:20	3:50	3.83	2,205	0.60
4/1/12 9:55	4/1/12 14:00	4:05	4.08	0.25	0.06	4/1/2012 9:00	4/1/2012 14:20	5:20	5.33	1,283	0.42
5/14/12 3:50	5/14/12 9:40	5:50	5.83	0.21	0.04	5/14/2012 2:00	5/14/2012 9:00	7:00	7.00	821	0.17
10/1/12 9:05	10/1/12 17:15	8:10	8.17	0.21	0.03	10/1/2012 7:30	10/1/2012 17:10	9:40	9.67	979	0.20

Figure 4 - Storm depths and inflow volumes.

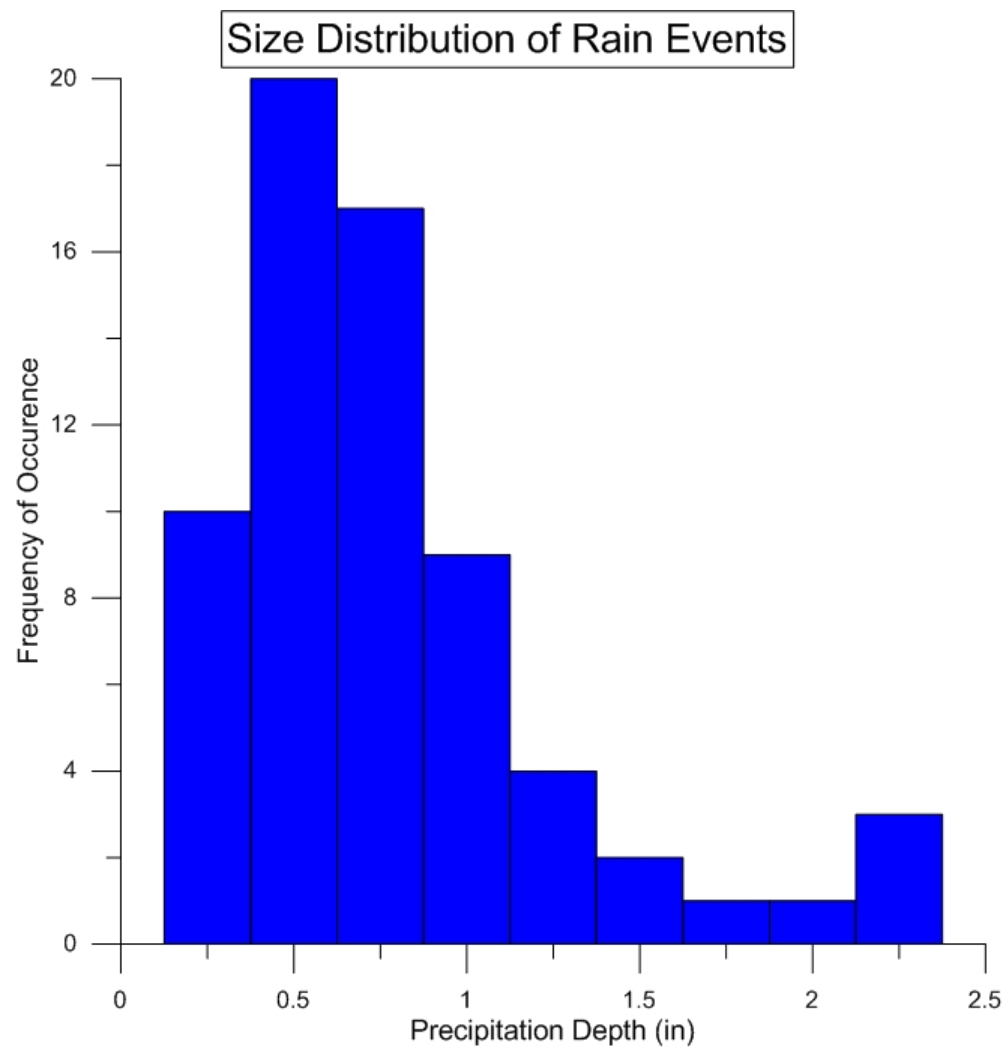


Figure 5: Distribution of precipitation events based on rainfall depth.

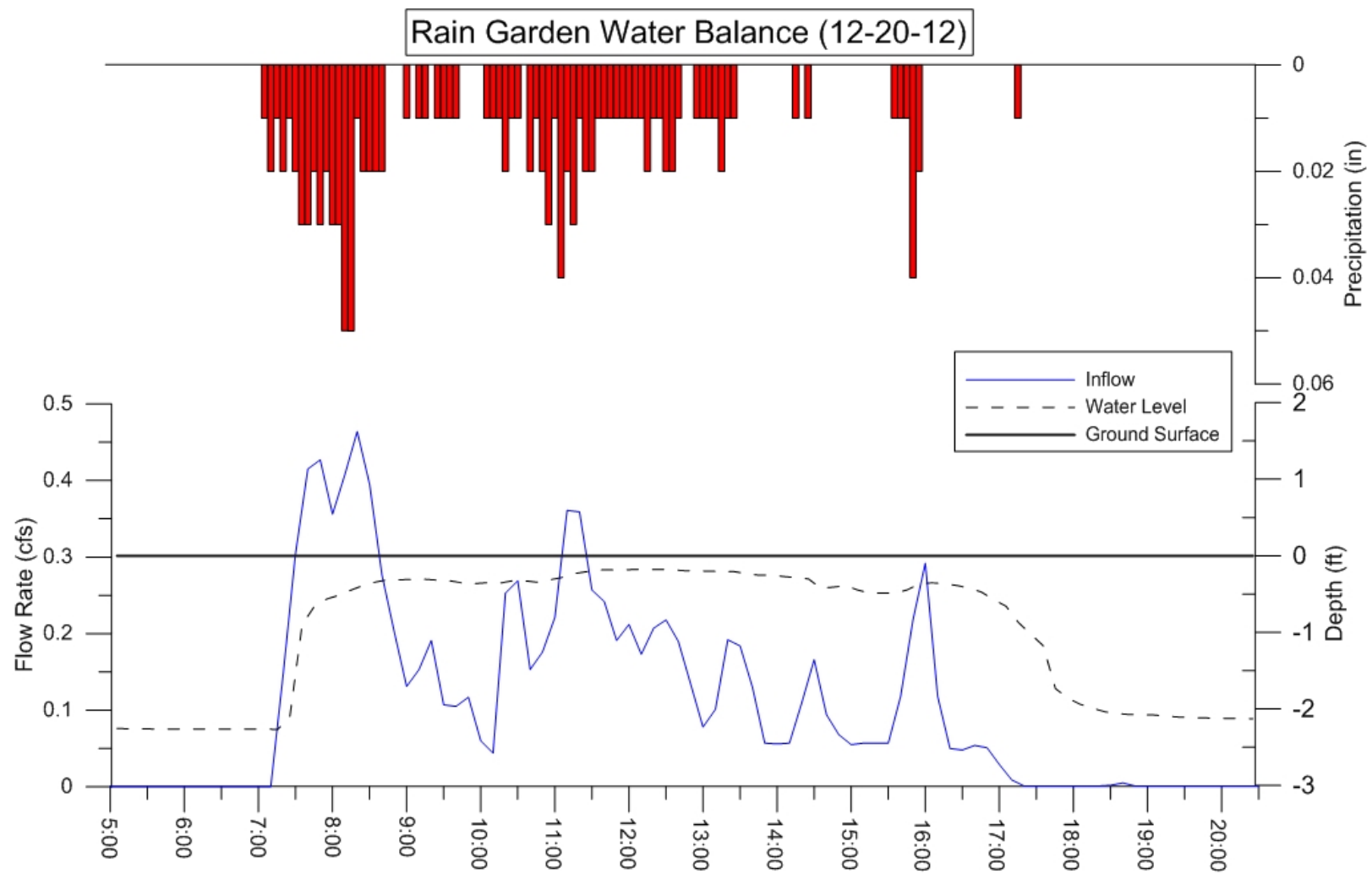


Figure 6: Example of rainfall, inflow, and water depth for an event on December 20, 2012.

Development and calibration of biomarkers in native sunfish for use in detection of endocrine active compounds

Basic Information

Title:	Development and calibration of biomarkers in native sunfish for use in detection of endocrine active compounds
Project Number:	2012KY201B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 5th
Research Category:	Biological Sciences
Focus Category:	Ecology, Water Quality, Surface Water
Descriptors:	endocrine active compounds, water quality monitoring
Principal Investigators:	David Peyton, Ben Brammell

Publications

1. Johnson, Matthew, Ben Brammell, David Peyton, Ben Adams, and Andrew Wigginton, 2013, Gene Expression in Sunfish as a Biomarker of Contaminant Exposure, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 85
2. Johnson, Matthew, Ben Brammell, David Peyton, Ben Adams, and Andrew Wigginton, 2012, Gene Expression in Sunfish as a Biomarker of Contaminant Exposure, Kentucky Academy of Science Annual Meeting, October 19-20, 2012, Eastern Kentucky University, Richmond, KY
3. Kirtland, Marina, Meredith Eckstein, David Peyton, Ben Brammell, Andrew Wigginton, and Scott Lynn, 2012, Gene Expression in Native Sunfish as a Biomarker of Contaminant Exposure, Kentucky Academy of Science Annual Meeting, October 19-20, 2012, Eastern Kentucky University, Richmond, KY
4. Kirtland, Marina, Meredith Eckstein, David Peyton, Ben Brammell, Andrew Wigginton, and Scott Lynn, 2012, Gene Expression in Native Sunfish as a Biomarker of Contaminant Exposure, Southern Regional Honors Conference, April 4-6, 2012, Louisville, KY
5. Johnson, Matthew, 2013, Use of Biomarker Gene Expression in Contaminant Detection, Annual Undergraduate Research Presentation, Asbury University, Wilmore, KY

Development and Calibration of Biomarkers in Native Sunfish (*Lepomis spp.*) for Use in Detection of Endocrine Active Compounds

Problem and Research Objectives (NOTE: This is the same report as 2011KY213B)

Recent studies have described widespread incidences of anatomical and physiological alterations in fish linked to waterborne pollutants (1, 2; 3, 4). Observed alterations include occurrence of intersex fish (5), abnormal hormone levels (6), reduction in reproductive success (7), the presence of tumors (8), and abnormal liver to body weight ratios (9). These alterations have been demonstrated to result from pollutants that affect the hypothalamus-pituitary-gonad axis, collectively referred to as endocrine active compounds (EACs) (10). EACs include a number of prevalent water borne pollutants including organochlorine pesticides, polychlorinated biphenyls (PCBs), heavy metals, pharmaceuticals, and surfactants (5). Currently the effects of EACs in freshwater fish are most frequently observed through studies examining histology of the gonads (5), alterations in plasma hormone levels (11), or detailed studies examining fecundity and reproductive success (6). Although these studies provide great insight into the nature of contaminant effects they are expensive, time consuming, and require specialized techniques. Many contaminants interact not only with the endocrine system but also with other aspects of normal physiology, resulting in alterations of specific enzymes or proteins known as biomarkers (12). Monitoring the expression of biomarkers provides an alternative mechanism of contaminant detection that offers a number of advantages (12, 13, 14, 15, 16, 17).

Pollutants found in surface waters have frequently been observed to alter the health and reproductive status of resident fish populations (1, 2, 3; 4, 5). Intersex fish, a condition frequently attributed to contaminant presence, have been observed in Europe for over a decade (2) but recent studies reporting widespread observations of intersex fish in North America (5) highlight the need for a greater understanding of this phenomenon. Many contaminants interact not only with the endocrine system but also with other aspects of normal physiology, resulting in alterations of specific enzymes or other proteins known as biomarkers (12). The use of biomarkers in pollutant detection offers a number of advantages including the ability to quickly screen for a wide variety of compounds, the ability to detect contaminants of an intermittent nature, and the relatively limited skill set and facilities necessary to utilize biomarkers. In addition, advances in biotechnology have greatly enhanced both the sensitivity and convenience of quantifying biomarkers at the level of mRNA expression. Despite these clear advantages to biomarker utilization in contaminant detection, few studies have utilized biomarker expression in native North American fish species that actually reside in contaminated waters. This study supported development of tools necessary to utilize eight biomarkers in two widely distributed fish species using real-time quantitative PCR. The collection site for this project has highly characterized contaminant levels and has been the location of multiple previous studies utilizing similar biomarkers in a well-characterized model (15, 21). Therefore, at the completion of this project, a large volume of pre-existing data will facilitate a thorough evaluation of gene-expression analysis as a method of contaminant detection and monitoring.

Previous work has provided a well documented distribution of metal and PCB contamination at the sites to be used in the proposed study (18, 19, 20). In addition, two recently conducted studies examined alterations in a number of biomarker genes in zebrafish (*Danio*

rerio), demonstrating the bioavailability of contaminants in these sites. One study conducted in the spring of 2009 used water collected from the same sites that will be utilized in the proposed study and exposed zebrafish to this water in the laboratory. Both catalase and cytochrome P4501A (CYP1A) were altered in contaminated sites, and the CYP1A response seemed to be a direct consequence of the presence of polychlorinated biphenyls (15). A study conducted in the fall of 2010 exposed caged zebrafish to water in the same sites, again documenting the presence of elevated CYP1A expression correlating to PCB contamination (22). The current study provides a unique opportunity to examine expression of biomarkers in two previously uncharacterized species in a system in which both contaminant levels and biomarker response in a model organism have been recently documented. While using biomarkers in model organisms such as zebrafish can be an effective method, it necessitates complex studies whereby the organisms are either exposed in cages or water from the sites is returned to the lab. Examination of biomarkers in widely distributed native fish increases the ease with which biomarker studies may be conducted and provides valuable information on the effects of contaminants on native organisms. The results of this study will facilitate the use of biomarkers in contaminant detection studies by providing tools and data necessary to use common fish to detect contaminants.

We examined the effect of contaminant exposure on three biomarkers at the level of mRNA expression in a species of sunfish (green sunfish, *Lepomis cyanellus*) widely distributed throughout North America. The results of this study provide data essential to the utilization of biomarkers for contaminant detection using native species and in addition will provide tools, in the form of nucleic acid sequences, that are universally available to researchers and enable the widespread use of biomarkers for contaminant detection.

The specific objectives of this research were to:

1. Acquire nucleic acid sequences from green and longear sunfish that represent several established biomarker genes known to be altered in fish following exposure to various contaminants: cytochrome P4501A, metallothionein, and glutathione S-transferase (2, 12).
2. Utilize these sequences to quantify biomarker gene expression in fish collected in both reference areas and areas receiving industrial effluent (and known to contain relatively high levels of both organic and metal contaminants).

Methodology

Study Site - The Paducah Gaseous Diffusion Plant (PGDP) is a uranium enrichment facility located in western KY. The streams surrounding the PGDP have a long and well documented history of contamination by metals and PCBs and both longear sunfish and green sunfish, the focus of this study, are abundant in these watersheds (18,19). In addition, recent studies document the presence and bioavailability of both PCB and metal contaminants in these streams (15).

Field collections - Longear and green sunfish were collected from both contaminated and reference sites surrounding the PGDP. Gill and liver tissue were removed and flash frozen in liquid nitrogen for transport back to the lab.

RNA extraction and cDNA generation - Liver tissue was removed from storage in the lab and a subsample removed and transferred to Ambion's lysis buffer for total RNA isolation. First strand cDNA was generated using Applied Biosystem's High Capacity RNA-to-cDNA Kit according to the manufacturer's instructions.

Gene sequencing - RACE PCR was used to amplify segments of cDNA that was subsequently checked using gel electrophoresis for expected length. Candidate fragments were ligated to the P-GEM-T Easy vector (Promega, Inc.) and transformed into JM-109 *E. coli* cells. Primers for real-time PCR were designed for expression analysis.

Real-time PCR - Quantification of mRNA in the livers was conducted by real-time PCR using the iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc., Tokyo, Japan) and iCycler iQTM Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.).

Principal Findings and Significance

The sequence of three genes frequently utilized as indicators of contaminant exposure were obtained (Table 1 and 2, Figure 3). Partial coding sequences for sequences for cytochrome P4501A, metallothionein, and glutathione S-transferase were obtained for green sunfish (*Lepomis cyanellus*) and metallothionein and glutathione S-transferase were obtained for longear sunfish (*Lepomis megalotis*). The sequences are shown in Tables 1 and 2. The acquisition of these sequences makes the utilization of these commonly used biomarkers possible in gene expression studies in both of these fish species found throughout most of North America.

Gene expression was quantified in green sunfish collected in clean and contaminated sites around the PGDP in western Kentucky (Figure 1). Relative expression of GST was greatest at BC8 and lowest at BC1A (the reference location), however, no significant differences were observed between sites. Gene expression of MT was lowest at BC5 and relatively high at BC1A although again, no significant differences were observed between sites. CYP1A gene expression was greatest at BC5 and lowest at BC1A with no significant differences observed.

Several studies collectively support the conclusion that *Lepomis* species may be relatively insensitive to polychlorinated biphenyls and have an inherent resistance to induction of CYP1A and other pollutant sensitive genes, rather than an acquired resistance as has been frequently observed in other species. In this study, there were no significant differences in green sunfish (*Lepomis cyanellus*) gene expression (GST, MT, and CYP1A) between the contaminated and reference sites. A similar study (15) found no significant difference in gene expression between reference and contaminated sites were observed in four of six pollutant sensitive genes examined in zebrafish (*Danio rerio*) caged in waters surrounding the Paducah Gaseous Diffusion Plant. However, that study did differ from this one in that significant differences were observed in CYP1A expression in caged zebrafish. This difference is perhaps reflective of the reported limited sensitivity of *Lepomis* species to PCB type inducers (16). This study provides data essential to evaluating the response of native fish to contaminants found in their environment as well as information perhaps relevant to the trophic transfer of contaminants from aquatic to terrestrial systems resulting from fish able to persist in relatively high contaminant levels.

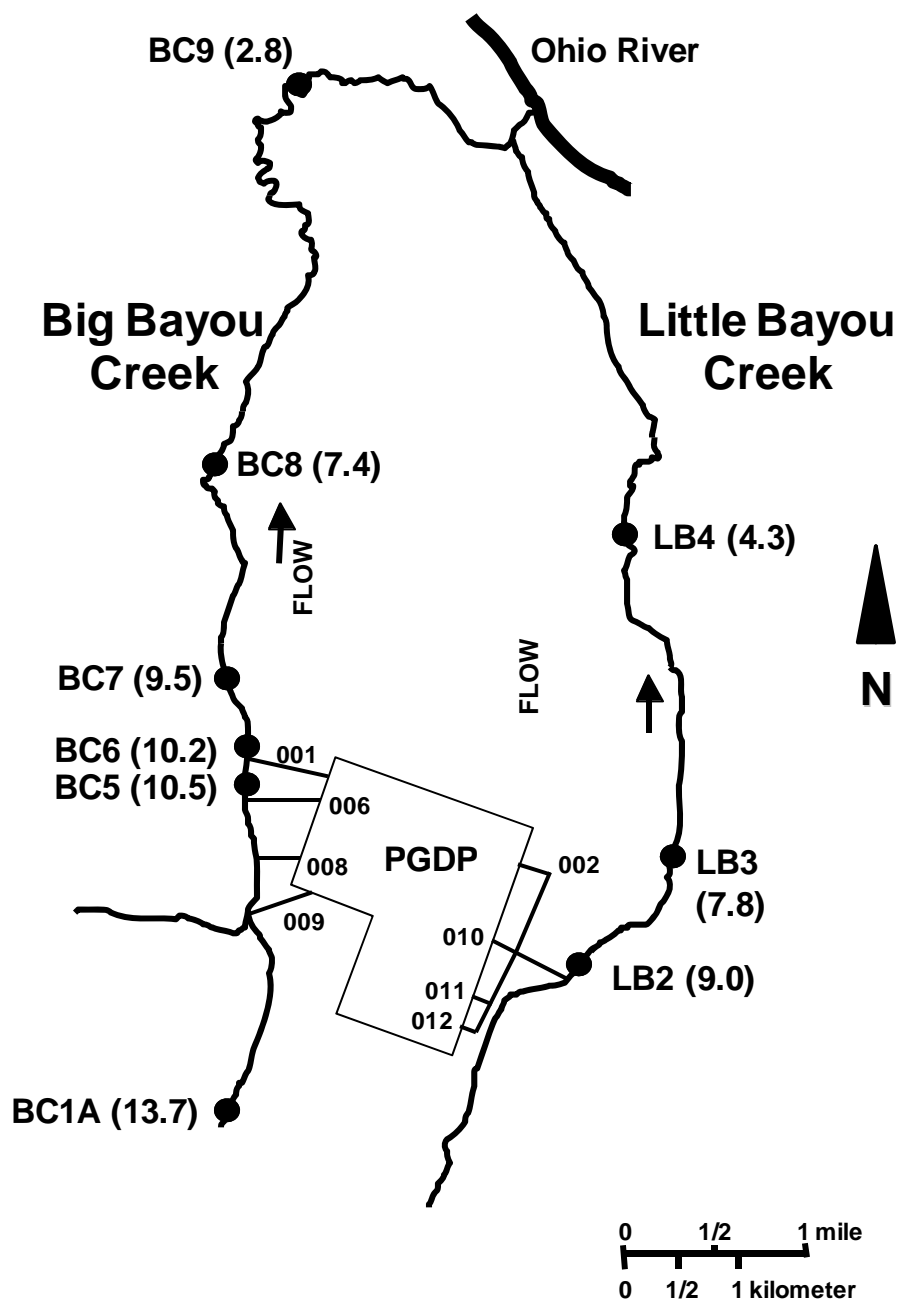


Figure 1. Map of study sites surrounding the Paducah Gaseous Diffusion Plant located in Western Kentucky.

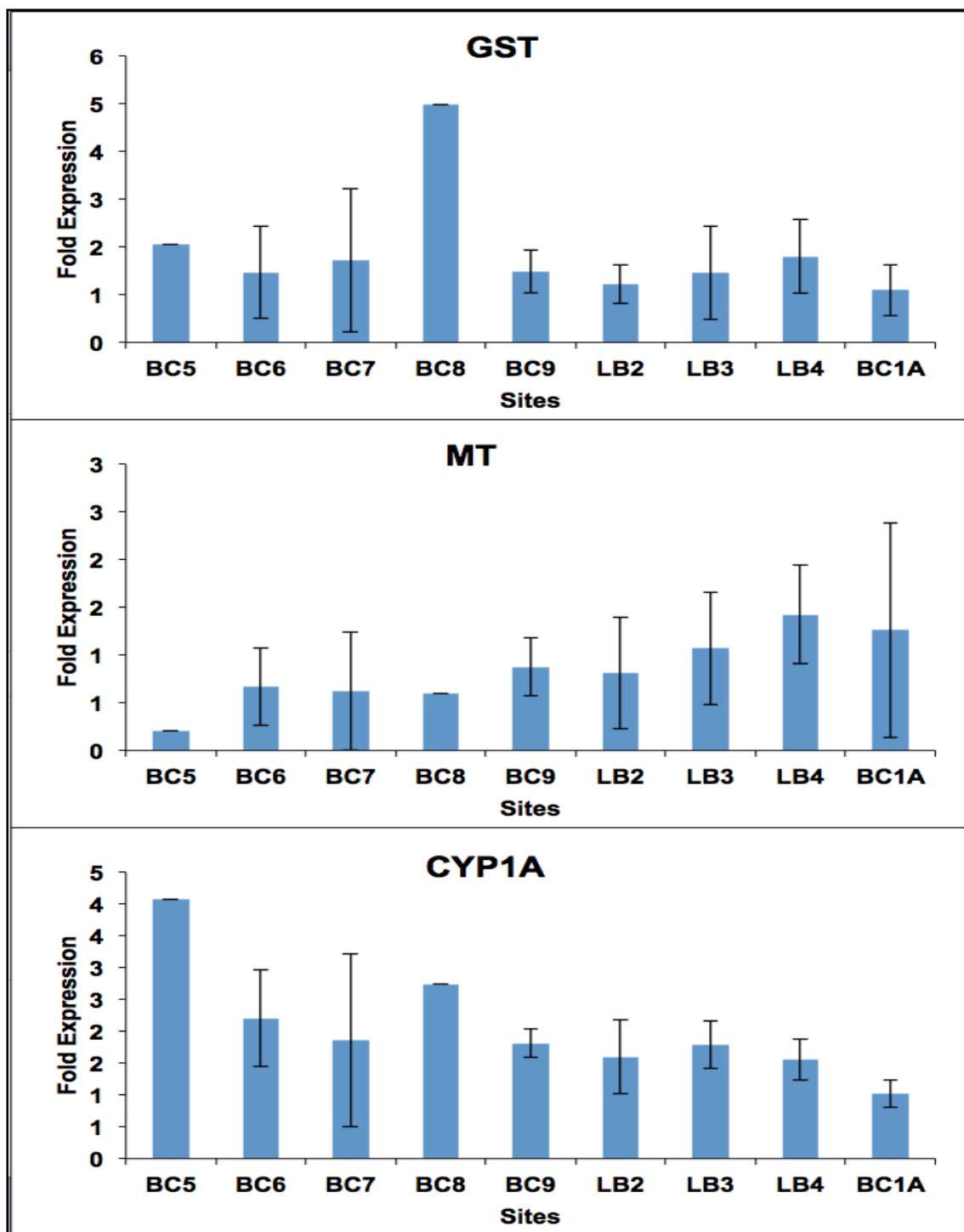


Figure 2. Gene expression (glutathione S-transferase (GST), cytochrome P4501A (CYP1A), and metallothionein (MT) in green sunfish (*Lepomis cyanellus*) collected from Bayou Creek (BC) and Little Bayou Creek (LB) around the Paducah Gaseous Diffusion Plant in Paducah, KY (McCracken County).

A. Score = 123 bits (136), Expect = 2e-33
Identities = 94/111 (85%), Gaps = 0/111 (0%)
Strand=Plus/Plus

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Query 16  TGCACCACCTGCAAGAAGAGCTGCTGCTCATGCTGCCCATCAGGCTGCAGCAAGTGTGCC 75
          |||
Sbjct 73  TGTACTACCTGCAAGAAGAGTTGTTGTTCTTGCTGCCCATCTGGTTGCAGCAAGTGTGCC 132

Query 76  TCTGGCTGCGTGTGCAAAGGGAAGAAATGTGACACCAGCTGCTGTCAAGTGA 126
          |||
Sbjct 133 TCTGGCTGCGTGTGCAAAGGCAATTCCTGTGGCTCCAGCTGCTGTCAATGA 183

Score = 25.8 bits (55), Expect = 4e-05, Method: Compositional matrix adjust.
Identities = 13/17 (77%), Positives = 14/17 (83%), Gaps = 0/17 (0%)

Query 25  ASGCVCKGKKCDTSCCQ 41
          ASGCVCKG C +SCCQ
Sbjct 44  ASGCVCKGNSCGSSCCQ 60

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B. Score = 652 bits (722), Expect = 0.0
Identities = 393/414 (95%), Gaps = 0/414 (0%)
Strand=Plus/Plus

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Query 1  ATCCCCGACTGCTCGGCTGAGAAAGCACTGATGTACCAGCGCATGTTTGAGGGTCTCACG 60
          |||
Sbjct 265 ATCCCCGACTGCTCGGCTGAGAAAGCACTGATGTACCAGCGCATGTTTGAGGGTCTCACG 324

Query 61  CTCACCCAGAAAATGGCTGATGTTATCTACTACAAGGTCCCTGAGGGAGAGAGA 120
          |||
Sbjct 325 CTCACCCAGAAAATGGCTGATGTTATCTACTACAAGGTCCCTGAGGGAGAGAGA 384

Query 121 CACGACTCTGCTGTGAAGAGAAACAGAGAGGCCCTGAGTGTGAGGTCAAACGTGGGAG 180
          |||
Sbjct 385 CACGACTCTGCTGTGAAGAGAAACAGAGAGCGTCTGAGTGTGAGGTCAAGCTGTGGGAG 444

Query 181 GGATACCTGCAGAAGGCATCAGGCCCTTCTCTGGCAGGAAAGAACTTTTCTCTGGCTGAT 240
          |||
Sbjct 445 GGATACCTGCAGAAGGCATCAGGCTCTTCTCTTGCAGGAAAGAACTTTTCTCTGGCTGAT 504

Query 241 GTGATCGTTTATCCATCCATCGCTTATATCTTCCACTTTGGGTTATGTGAAGAGCGTTAC 300
          |||
Sbjct 505 GTGACGGTTTATCCATCTATCGCTTATCTCTTCCATTTTGGGTTGTGTGAAGAGCGTTAC 564

Query 301 CCTAAACTGGCAGCTTACTATAACGCCAATAAGGAGAGACCCAGCATCAAAGCCACATGG 360
          |||
Sbjct 565 CCTAAACTGGCAGCTTACTATAACTCCAATAAGGACAGACCCAGCATCAAAGCCACATGG 624

Query 361 CCTCCTTCTCTGGCTGGAGAGCTCACAGGGACAAGACCAACTGAAAGACATTTGA 414
          |||
Sbjct 625 CCTCCTACCTGGCTGGAGAACCACAGGGACAAGACCAACTGAAAGACATTTGA 678

Score = 267 bits (683), Expect = 6e-77, Method: Compositional matrix adjust.
Identities = 124/136 (92%), Positives = 130/136 (96%), Gaps = 0/136 (0%)

Query 1  IPDCSAEKALMYQRMFEGTLTLQKMDVYYNWKVPEGERHDSAVKRNREALSAEVKLWE 60
          |||
Sbjct 89  IPDCSAEKALMYQRMFEGTLTLQKMDVYYNWKVPEGERHDSAVKRNRLVLSAEVKLWE 148

Query 61  GYLQKASGFFLAGKNFSLADVYPYIAYIFHFGLCCEERYPKLAAYYNANKERPSIKATW 120
          |||
Sbjct 149 GYLQKASGSFFAGKNFSLADVTYPYIAYLFFHFGLCCEERYPKLAAYYNSNKDRPSIKATW 208

Query 121 PPSWLESSQGQDQLKD 136
          PP+WLE+ QGQDQLKD
Sbjct 209 PPTWLENPQGQDQLKD 224

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Figure 3. The nucleotide and protein alignments (A) of the longear sunfish metallothionein-2 (query) and zebrafish (*Danio rerio*) metallothionein-2 (subject) gene and protein, and (B) the longear sunfish (*Lepomis megalotis*) glutathione-S-transferase (query) and largemouth bass (*Micropterus salmoides*) glutathione-S-transferase gene and protein.

Table 1. Partial sequences obtained for longear sunfish (*Lepomis megalotis*).

MT:TGCGCAAAGTGTGCTCCTGCACCACCTGCAAGAAGAGCTGCTGCTCATGCTGCCCATCAGG
CTGCAGCAAGTGTGCCTCTGGCTGCGTGTGCAAAGGGAAGAAATGTGACACCAGCTGCTGTC
AGTGA

GST:ATCCCCGACTGCTCGGCTGAGAAAGCACTGATGTACCAGCGCATGTTTGAGGGTCTCAC
GCTCAGCAAGTGTGCCTCTGGCTGCGTGTGCAAAGGGAAGAAATGTGACACCAGCTGCTGTC
AGTGA
CACGACTCTGCTGTGAAGAGAAACAGAGAGGGCCCTGAGTGCTGAGGTCAAAGTGTGGGAGG
GATACCTGCAGAAGGCATCAGGCCCTTTCTGGCAGGAAAGAACTTTTCTTGGCTGATGTG
ATCGTTTATCCATCCATCGCTTATATCTTCCACTTTGGGTTATGTGAAGAGCGTTACCCTAAA
CTGGCAGCTTACTATAACGCCAATAAGGAGAGACCCAGCATCAAAGCCACATGGCCTCCTTC
CTGGCTGGAGAGCTCACAGGGACAAGACCAACTGAAAGACATTTGA

Table 2. Partial sequences obtained for *Lepomis cyanellus* (green sunfish).

MT:TGCGCAAAGTGTGCTCCTGCACCACCTGCAAGAAGAGCTGCTGCTCATGCTGCCCATCAGG
CTGCAGCAAGTGTGCCTCTGGCTGCGTGTGCAAAGGGAAGAAATGTGACACCAGCTGCTGTC
AGTGA

GST:ATCCCCGACTGCTCGGCTGAGAAAGCACTGATGTACCAGCGCATGTTTGAGGGTCTCAC
GCTCAGCAAGTGTGCCTCTGGCTGCGTGTGCAAAGGGAAGAAATGTGACACCAGCTGCTGTC
AGTGA
CACGACTCTGCTGTGAAGAGAAACAGAGAGGGCCCTGAGTGCTGAGGTCAAAGTGTGGGAGG
GATACCTGCAGAAGGCATCAGGCCCTTTCTGGCAGGAAAGAACTTTTCTTGGCTGATGTG
ATCGTTTATCCATCCATCGCTTATATCTTCCACTTTGGGTTATGTGAAGAGCGTTACCCTAAA
CTGGCAGCTTACTATAACGCCAATAAGGAGAGACCCAGCATCAAAGCCACATGGCCTCCTTC
CTGGCTGGAGAGCTCACAGGGACAAGACCAACTGAAAGACATTTGA

CYP1A:GACACCTGTGTCTTCATCAATCAGTGGCAAATCAACCATGATCCGGAGTTGTGGAAA
GATCCGTCTTCCTTCAACCCAGATCGCTTCCTGAGTGCTGATGGCACTGAGCTCAACAAGCTG
GAGGGGGAGAAAGGTTACAACCTTTTGGCTTGGGAAAGCGGCGCTGCATCGGCGAGGTCAATTG
CACGAAATGAAGTCTACCTCTTCTTGGCAATCATGGTCCAGAAGCTGCACTTCAGCGCAATT
CCTGGAGAGCCGCTGGACATGACCCAGAATACGGTCTCACAATGAAGCACAAACGCTGCA
ACCTGAGAGCCACGATGCGAGCGAGGGATCAGCAGTGAATGTATGAATGTGTTATTTTCAAT
GTACAATGTTTACTCAACAGGTGGTATGAGTTAACTCATAAAGTGGATGGGTTATAGTCA
GGTTAAGGGTTAGTGAAAGAAGCATCTCTCTCAGAATGTAAGGCACTGGATCCCGAAATGG
ATCCACAGAGCTACTGGCATTAAAGCAAATATGTGGATTTTGCTTGCTGCAGATTGTCAGAG
ATGCTTGGTTCTTATGAACCTATCTCTGTCATGTTTTTGGTTTACCAAGAGATATTCCTGCAC
ATGACTGGTGCTACTCAGTGAAGTATAAGGATATACTTGGTTCTCCTGTGTTGGGTTGTGTAG
TAAACTGTTCCCTTAAGAAGTTGTAAAACACACAGAGACTATGTTTTGTTTGTGTTTCAAC
CTCTGGGACAAACAGTATGCTTTACAGGATAGGCATTACTATTTAGACCACTGCAAACAACA
TTTTTCTCTTCTGTGACACA

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The combined effects of the herbicide atrazine and predator cues on larval dragonflies

Basic Information

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Descriptors:	predation risk, immune function, sublethal effects
Principal Investigators:	Claire A. Fuller

Publications

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The Combined Effects of the Herbicide Atrazine and Predator Cues on Larval Dragonflies

Problem and Research Objectives

Atrazine is one of the most widely used herbicides in the United States, where it is applied throughout the growing season to control weeds primarily in corn fields (Kiely et al. 2004, Rohr and McCoy 2010). Due to its widespread use, timing of application, and high runoff potential, atrazine is the most commonly found herbicide in surface waters and groundwater (Russo and Lagadic 2004; Barr et al. 2007). Typical surface water concentrations of atrazine are low (3-50 $\mu\text{g/L}$), however concentrations in agricultural wetlands frequently surpass 500 $\mu\text{g/L}$ (reviewed in Rohr and McCoy 2010). Although atrazine may be quickly removed from flowing waters, concentrations in lentic systems are likely to persist longer (Solomon et al. 2008).

Persistence of atrazine in aquatic systems typically leads to indirect and sublethal effects on aquatic wildlife. Examples include decreased food and habitat resources via removal or reduction of aquatic plants (Huber 1993), altered food web interactions, and/or sublethal effects (e.g. alteration of physiological processes, growth, and reproduction; EPA 2008). Past research shows that sublethal effects are relevant in most freshwater systems (Relyea and Edwards 2010). Some examples of sublethal atrazine effects include altered reproductive development and limb deformities in anurans (Hayes et al. 2003; Allran and Karasov 2001); reduced kidney function and gill damage in fish (Fisher-Scherl et al. 1991; Alazemi et al. 1996); and increased susceptibility to viral infection in tiger salamander (Forson and Storfer 2006).

Previous toxicological studies using atrazine have focused on direct effects and single-species laboratory studies (Relyea and Edwards 2010), whereas the indirect and sublethal effects in community studies have focused on zooplankton and larval amphibians (e.g. DeNoyelles 1982, Boone and James 2003). Aquatic invertebrates are a fundamental component of aquatic communities, however they have been relatively neglected with respect to toxicological studies at the community level (Galloway and Depledge 2001). Community-level studies have shown that atrazine negatively affects aquatic invertebrates (e.g. Relyea and Hoverman 2008) however the mode by which it does so is less understood. Furthermore, few studies have attempted to couple the sublethal effects with natural processes such as predation to comprehend the impacts on life history traits (e.g. Campero et al. 2008). Therefore, research on the sublethal effects of atrazine on aquatic invertebrates in natural environments is imperative to understand the pervasive effects of exposure to individuals and potential community alterations.

Recent work in our lab suggests that atrazine negatively affects cannibalistic behaviors and immune parameters in dragonfly larvae (St. Clair 2011), however there is still a great deal of uncertainty as to the effects of atrazine when combined with community interactions in a naturalistic environment. The objective of the current research was to determine the combined effects of sublethal atrazine exposure and

predation risk on immune investment, growth rate, and energy storage, using larval dragonflies as a representative aquatic invertebrate. This study will lead to a greater understanding of the extent of atrazine interactions with natural stress (i.e. predation risk) to shape life history and immune function in aquatic invertebrates.

Methodology

Larva collection: Dragonfly larvae (*Ladona deplanata* and *Anax junius*) were collected using aquatic D-frame dip nets from lakes and ponds at Land-Between-the-Lakes National Recreation Area and Hancock Biological Station. Species were identified in the field and brought to Hancock Biological Station (HBS).

Experimental set-up: Experimental mesocosms were first set up in early spring 2012 at the Hancock Biological Station. Because weather events disrupted the mesocosms and diluted atrazine levels, we set up the experiment again in late summer 2012. Preliminary data presented below are from the second, successful, experiment. Each mesocosm was filled with 1000 L of tap water and allowed to de-chlorinate for two weeks before adding leaf litter and inoculating with zooplankton. Zooplankton was collected from the same ponds as dragonflies. Zooplankton from various ponds were mixed and added in 2L aliquots to all mesocosms to initiate aquatic food webs. Water quality (e.g. pH, temp, and dissolved oxygen) and standardized density measurements were taken at each larva collecting location during collection events to facilitate natural density calculations of *L. deplanata* and to ensure that experimental mesocosms mirrored natural habitats in terms of water quality and larval density.

The experiment was a split plot factorial design with randomly assigned treatments. Factors were atrazine (presence or absence) and predators (presence or absence). Thus, the 4 treatments consisted of 1) predators and atrazine absent (control), 2) predators only (no atrazine), 3) atrazine only (no predators), 4) both predators and atrazine. Groups of six *L. deplanata* were housed in mesh-covered cages, each containing a clear plastic predator cup with holes to allow for the exchange of visual and chemical cues. Each mesocosm had four *L. deplanata* cages placed equidistance apart, and predator treatments had one *A. junius* cup per cage. Treatments were replicated six times for a total of 24 mesocosms and 96 cages yielding 576 individual *L. deplanata* and 48 predators. The target atrazine level was 80 µg/L. Atrazine concentrations were tested once a month to ensure uniform exposure and re-dosed when necessary.

Outcome variables: Prior to the experiment, individuals were marked and body measurements recorded to allow calculation of individual growth rates over the length of the experiment. Individuals were destructively sampled at 5, 15, 30, and 45 days, upon which each was measured and immunocompetence was measured by assaying two common indicators of immune function from the hemolymph: hemocyte load and phenyloxidase (PO) activity. In addition to immune parameters, we are currently performing fat extraction analyses to measure fat storage, an indicator of body condition and possible source of tradeoff with immunity and/or growth.

Data analysis: Preliminary analysis of data at each sampling day was performed using two-way analysis of variance (ANOVA) with factors predation and atrazine, and response variables, PO activity and hemocyte count. For each day (5, 15, 30 and 45), treatment effects (atrazine and predation) were tested on growth as a binomial response (yes, no) using generalized linear models with a logit link function.

Principal Findings and Significance

After five days of exposure, no significant treatment effects or interaction were detected on response variables (hemocyte, $F_{1,92}=0.82$, $p=0.37$; PO activity, $F_{1,89}=0.38$, $p=0.54$; growth, $\chi^2=0.00$, $p=1.0$, $d.f.=1$). Similarly, after fifteen days exposure, there was no significant treatment or interaction effects on individual growth ($\chi^2=0.32$, $p=0.57$, $d.f.=1$), however predators had a significant negative effect on growth ($\chi^2=4.04$, $p=0.04$, $d.f.=1$). At fifteen days, two-way ANOVAs showed a significant interaction on hemocyte count ($F_{1,90}=5.68$, $p=0.02$; Fig. 1), only the main effect predator exposure resulted in significantly decreased PO activity (atrazine X predator, $F_{1,87}=1.71$, $p=0.19$; predator, $F_{1,87}=5.17$, $p=0.02$; Fig. 2).

At thirty days, there was no effect of atrazine on response variables measured (hemocyte, $F_{1,78}=0.3485$, $p=0.5567$; PO activity, $F_{1,79}=1.6959$, $p=0.196611$; growth, $\chi^2=0.3076$, $p=0.57918$, $d.f.=1$); with predators having a significant negative effect on growth ($\chi^2=4.2242$, $p=0.03985$, $d.f.=1$). Contrary to the effect at fifteen days, there was a significant increase in PO activity in the predator treatments (PO activity, $F_{1,79}=7.182$, $p=0.008$, Fig. 3). By forty-five days of exposure, there was no significant treatment or interaction effect on response variables measured (hemocyte, $F_{1,55}=0.6913$, $p=0.4093$; PO activity, $F_{1,47}=0.6270$, $p=0.4324$; growth, $\chi^2=0.04690$, $p=0.8286$, $d.f.=1$).

Our results show that after five days of exposure, sublethal atrazine exposure did not effect the life history variables measured in larval dragonflies, however, effects on immune function were evident after fifteen days. The effect of atrazine exposure depends on the immune component measured, as effects were evident on hemocyte count but not PO activity. Contrary to previous research with aquatic macroinvertebrates (aquatic snails; Russo and Lagadic 2000; Sandland and Carmonsini 2006), we found that atrazine suppressed circulating hemocytes in dragonfly larvae. The significant interaction of both treatment effects indicate that when both stressors are combined, dragonflies are not responding to predation risk as expected, rather atrazine may be interfering with the ability to detect predators and therefore larvae are maintaining hemocyte loads near normal levels (Luring and Scheffer 2007).

The relatively short-term effect (i.e. 15 days) of sublethal atrazine concentrations on dragonfly life history traits is consistent with previous research with both vertebrates (e.g. Brodtkin et al. 2007; Christin et al. 2007) and invertebrates (e.g. Sandland and Camosini 2006). The lack of atrazine effects on response variables measured indicates that sometime after fifteen days of exposure, larval dragonflies seem to acclimate to exposure. This is likely given that this species (*L. deplanata*) is relatively robust and can tolerate poor water quality parameters. Given that this experiment was performed in the

fall as dragonflies are building up reserves to overwinter, individuals may be allocating resources to other life-history traits such that atrazine may not be an important immune stressor during this time. Furthermore, these short term effects on dragonflies are important to consider along with the timing of final atrazine application and potential release of soil-bound atrazine into aquatic systems during fall precipitation events, as may occur during drought years. Thus, late-season pulses could have important implications for overwinter survival and ability to metamorphose in the following spring.

In this study, predation risk showed suppression of both immune parameters and growth at fifteen days. Interestingly, predators caused an increase in PO activity at thirty days. This change in PO activity may be linked to phenology; as dragonflies are preparing for winter, the risk of predators may be triggering an up-regulation of immune function to ensure over-winter survival. As long-term fat storage is required for winter survival (Lee and Denlinger 1991), and is reduced in predator-stressed odonates (damselflies; Stoks et al. 2005), ongoing analyses of dragonfly lipids may illustrate a life-history tradeoff between investment in immunity and energy storage. Overall, fat storage is an important component of odonate life-history, positively correlating with increased survival, successful metamorphosis, and mating success (reviewed in Stoks and Cordoba-Aquilar 2012), thus our analysis of fat storage has the potential to clarify the mechanism behind differences in the life history traits measured in this experiment.

Combined with previous research in our lab, this experiment demonstrates that atrazine does affect immune function in larval dragonflies, however the effect is variable and seems to depend on the response variable measured. Thus, future experiments should be designed to examine multiple response variables to best understand the sublethal effects of atrazine exposure. Because most research has focused on vertebrates, more studies examining sublethal effects on invertebrates are needed to identify the exact mechanisms impairing this group, as reduced fitness and altered species interactions could have pervasive ecosystem-level effects.

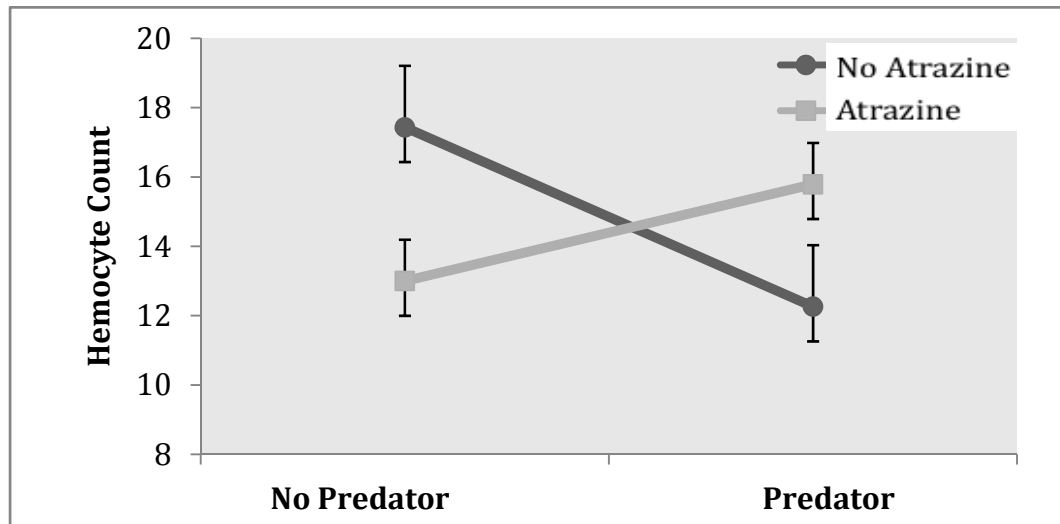


Figure 1. Significant interaction of treatment effects on mean hemocyte count. Atrazine significantly reduced hemocytes without predators; predators significantly reduced hemocyte count in atrazine-exposed larvae.

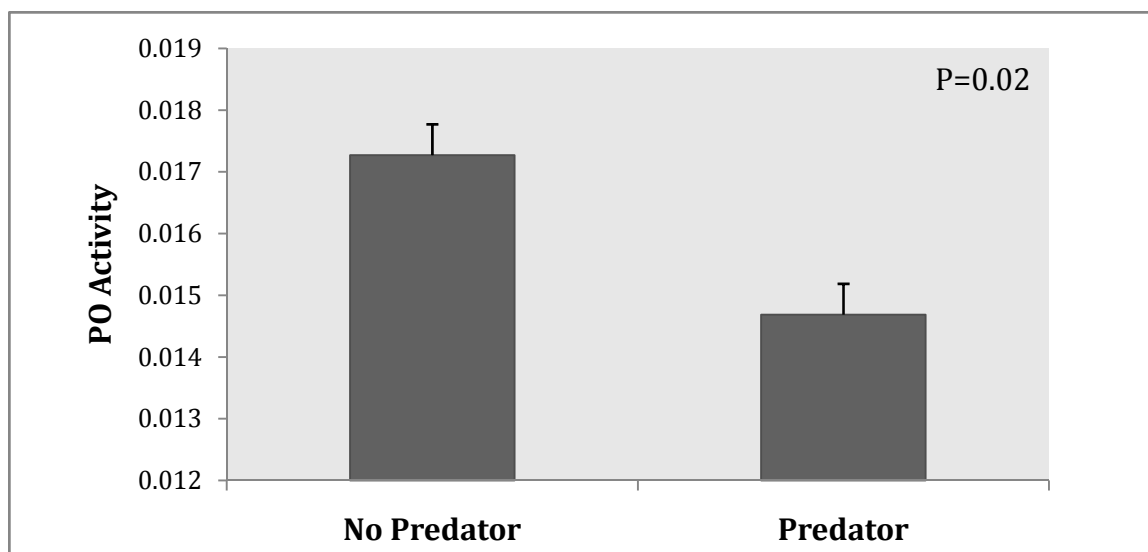


Figure 2. Phenoloxidase activity was significantly lower in larval dragonflies exposed to predators at day 15.

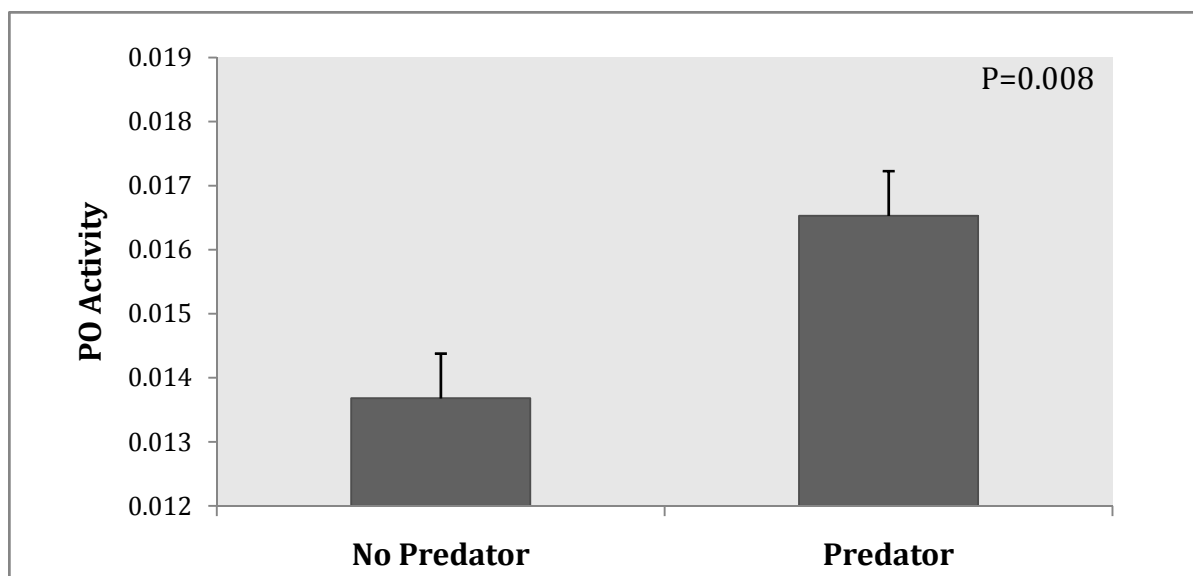


Figure 3. Phenoloxidase activity was significantly higher in predator treatments at 30 days.

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Occurrence of synthetic estrogen in surface water, source water, and drinking water in the Barren River watershed

Basic Information

Title:	Occurrence of synthetic estrogen in surface water, source water, and drinking water in the Barren River watershed
Project Number:	2012KY203B
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Congressional District:	KY 2nd
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Focus Category:	Water Quality, Acid Deposition, Surface Water
Descriptors:	wastewater, seasonal trends, EPA Method 539
Principal Investigators:	Ritchie Taylor

Publications

1. Grigsby, Roni, J. Eagleson, and Ritchie Taylor, 2013, Occurrence of Synthetic Estrogen in Surface Water, Sourcewater, and Drinking Water in the Barren River Watershed, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p. 101
2. Grigsby, Roni, J. Eagleson, and Ritchie Taylor, 2013, Occurrence of Synthetic Estrogen in Surface Water, Sourcewater, and Drinking Water in the Barren River Watershed, in Proceedings of the 2013 Western Kentucky University Student Research Symposium, Bowling Green, KY

Occurrence of Synthetic Estrogen in Surface Water, Sourcewater and Drinking Water in the Barren River Watershed

Problem and Research Objectives

Pharmaceuticals, estrogenic compounds and their metabolites have been confirmed in surface waters, including rivers and streams (Brooks, Riley, & Taylor, 2006; Zhang, Zhang, Zhang, Luo, & Yan, 2012). The primary source of these compounds was wastewater treatment plant (WWTP) effluents (Clouzot, Marrot, Doumenq, & Roche, 2008). Other studies have indicated that activities such as land application of dairy lagoon waters may be additional sources of contamination (Zhang, Li, Yates, & Bradford, 2012). Occurrence of estrogenic compounds in the environment is of concern because of the potential to affect human and ecosystem health (Caldwell, Caldwell, & Mastrocco, 2010). Research conducted on estrogens has shown that conventional treatment in WWTPs does not remove these compounds (Clouzot, Marrot, Doumenq, & Roche, 2008). Effluents from WWTPs represent a constant source of prescribed and endogenous estrogens due to excretion and flushing into wastewater systems.

Numerous laboratory studies have been conducted to document the effects of specific estrogens on fish and other aquatic organisms (Caldwell, et al., 2008). Barber et al. (2012; Barber, Vajda, Douville, Norris, & Writer, 2012) determined that effects on fish exposed to endocrine-disrupting chemicals could be reduced by improved wastewater treatment. However, few studies have focused on the risk to human health. Caldwell et al. (2010) performed a risk assessment of drinking water exposure. Results of this study focused on predicted concentrations of estrogens in drinking water instead of measured concentrations because few studies have measured estrogen concentrations in United States drinking water (Caldwell, Caldwell, & Mastrocco, 2010).

Due to the lack of data on the occurrence of estrogenic compounds in rural surface waters, sourcewaters and drinking water, limited information is available to assess environmental exposures to these compounds in rural regions of the United States. Likewise, very few studies document seasonal trends. Furthermore, only a few studies exist on the occurrence of specific pharmaceuticals in surface waters of Kentucky and these studies are not found in the published literature. Therefore, the primary objective of this study was to assess the occurrence of synthetic estrogen, specifically 17 α -ethinyl estradiol in surface water, sourcewater, and drinking water in the Barren River Watershed. A secondary objective was to evaluate the seasonal trend of occurrence. Finally, a tertiary objective was to assess the use of EPA Method 539, a method for determination of hormones in drinking water by solid phase extraction (spe) and liquid chromatography electrospray ionization tandem mass spectrometry (lc-esi-ms/ms), for use in extracting and measuring 17 α -ethinyl estradiol in sourcewater, surface water, and drinking water (Smith, Zaffiro, Zimmerman, & Munch, 2010). Based on these objectives the research questions are:

- Do 17 α -ethinyl estradiol and other estrogenic compounds occur in sourcewater, surface water, and drinking water in the Barren River Watershed?

- Do seasonal trends exist in the occurrence of estrogenic compounds in the Barren River Watershed?
- Can EPA Method 539 be used to extract and measure estrogenic compounds from surface water, sourcewater and drinking water samples collected from the Barren River Watershed?

The purpose of this study was to document the occurrence and seasonal trend of estrogenic compounds, specifically 17 α -ethinyl estradiol, in the Barren River Watershed of Kentucky, including the drinking water supply of Bowling Green, KY. This project was intended to be a catalyst for further research on exposure of humans and aquatic biota to estrogenic compounds in Kentucky's surface water, groundwater and drinking water supplies. Future research will use this baseline data to develop objectives that provide a more complete environmental exposure assessment. A primary goal was to begin to differentiate the various sources of estrogens in the Barren River Watershed, such as WWTP effluent or nonpoint sources.

Methodology

To assess the occurrence of estrogenic compounds in drinking water and surface water in the Barren River Watershed, EPA Method 539 was evaluated (Smith, Zaffiro, Zimmerman, & Munch, 2010). A series of samples were collected from Barren River upstream from the raw water intake for Bowling Green, KY, from a drinking water source in Bowling Green, and downstream from Bowling Green. The samples were collected from September 2012 - December 2012. In this manner, samples were collected during times when stormwater was present and during the low flow season, when the greatest concentrations were hypothesized to occur. Sampling and analysis were completed to obtain baseline data for concentrations of estrogenic compounds.

Synthetic estrogen was analyzed by modification of EPA method 539 and LC-ESI-MS/MS (Smith, Zaffiro, Zimmerman, & Munch, 2010). Significant time resources were utilized in modification of the method to adjust to the various sample matrices. Analyses were conducted at the Environmental Research Training Laboratory (ERTL) of the University of Kentucky. Quality assurance and quality control were administered by ERTL laboratory personnel. To evaluate this method, samples were analyzed from the various water matrices to assess accuracy and precision. Field blanks were used to assess contamination of samples via sample containers and preservation. Standards were used to assess calibration of the method and recovery in spiked samples.

In evaluation of EPA Method 539 (Smith, Zaffiro, Zimmerman, & Munch, 2010), it was discovered that extraction of samples took a significantly longer time than originally planned. Due to this deviation, EPA Method 539 (Smith, Zaffiro, Zimmerman, & Munch, 2010) was modified to a filtration and then an extraction step that performed well and reduced sample-processing times.

Principal Findings and Significance

The initial finding relevant to the determination of synthetic estrogen in the Barren River Watershed samples was that EPA Method 539 could be modified to recover estrogenic compounds in the sample matrices. Samples from each water matrix were spiked with surrogate compound, Ethinylestradiol- d_4 , to evaluate recovery through the filtration and extraction process. As compared to EPA method 539 (Smith, Zaffiro, Zimmerman, & Munch, 2010), acceptable surrogate recovery was achieved in all sample matrices based on mean percent recovery values (Table 1). Barren River upstream from Bowling Green, KY showed the least efficient recovery. However, all matrices were within the guidelines stated in EPA Method 539 (Smith, Zaffiro, Zimmerman, & Munch, 2010).

Table 1. Percent Surrogate Recovery for Modified Extraction Method

Sample Matrix	Mean	SD
Tap	94.5	7.7
Drakes Creek	98.25	21.5
Barren River Upstream	85.22	36
Barren River Downstream	98.75	22

Based on surrogate recovery values, the modified EPA Method 539 or EPA Method 539M, was deemed to produce acceptable results. Therefore, all samples were extracted and analyzed according to this modified method. In addition to Ethinyl Estradiol, and due to the ability to obtain standards for other estrogenic compounds, other estrogenic compounds were also analyzed in this study. These compounds included Equilin and Estrone. Additionally, Bisphenol A (BPA), a common chemical found in plastics, was analyzed for all water samples. BPA has been noted as a chemical of concern by the U.S. Food and Drug Administration (FDA) due to animal studies which indicate potential effects on the brain, behavior, and prostate glands in fetuses, infants and young children.

Results for analyses conducted with Method 539M are shown in Table 2. Samples for estrogenic compounds showed nondetects for all except one sample for each of the sampling sites. Detections were made in summer months, with most detections being made on the 8/31/2012 sampling date. Rainfall patterns in Bowling Green and stage data (ft) for the Barren River at Bowling Green indicated that several rain events had occurred in early fall, which is uncharacteristic. This may account for the onset of a dilution effect that resulted in nondetectable levels of estrogenic compounds in the samples. The

8/31/2012 sampling date was characterized by the lowest stage (ft) encountered during the study on the Barren River and Drakes Creek.

Maximum values detected for each compound occurred in Drakes Creek as follows: 8 ng/L of Equilin, 17 ng/L of Estrone, and 13 ng/L of Ethinyl Estradiol. The maximum value detected for BPA was 1,135 ng/L downstream from Bowling Green on the Barren River. All samples analyzed for BPA were found to be detects.

Although most samples analyzed for estrogenic compounds were found to be nondetects, there were detectable levels at the minimum stage date on 8/31/2012. Additionally, levels detected in Drakes Creek were greater than all other sites. Drakes Creek receives a greater percentage flow, by volume, of effluent from the Franklin, KY Wastewater Treatment Plant (WWTP). Previous research indicates that WWTP effluents contain estrogenic compounds at detectable levels (Clouzot, Marrot, Doumenq, & Roche, 2008). This research seems to agree with those findings.

Results from this study indicated that EPA Method 539 (Smith, Zaffiro, Zimmerman, & Munch, 2010), when modified with a filtration step, can be utilized to analyze estrogenic compounds in surface water and sourcewater samples. Although the method was efficient, detectable levels of estrogenic compounds were scarce in the samples collected during this study. However, detectable levels were found in a few samples.

Future research on the occurrence and distribution of estrogenic compounds in the Barren River Watershed should include a component to assess levels in WWTP effluents. Cooperation with local wastewater facilities will be invaluable to address potential seasonal variation and degradation. The current research did not find detectable levels of estrogenic compounds in drinking water samples. However, there is a potential that these compounds will occur in the sourcewater. Further studies should be conducted to continue to assess the risk of these compounds to human health.

Table 2. Summary of Barren River Watershed sample analyses results.

Sample Location	# of Samples (8/31 - 11/30 2012)	Equilin			Estrone			BPA			Ethinyl Estradiol		
		# of NDs	# of Detects	Maximum (ng/L)	# of NDs	# of Detects	Maximum (ng/L)	# of NDs	# of Detects	Maximum (ng/L)	# of NDs	# of Detects	Maximum (ng/L)
b	10	9	1	4	9	1	4	0	10	70	9	1	4
Barren River, Downstream from BG	10	6	4	3	6	4	6	0	10	1,135	9	1	10
Drinking Water (Tap at WKU)	10	10	0	ND	10	0	ND	0	10	293	10	0	ND
Drakes Creek (source of WWTP effluent)	8	7	1	8	7	1	17	0	8	218	7	1	13

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Field-scale water and bromide transport as affected by land use and rainfall characteristics

Basic Information

Title:	Field-scale water and bromide transport as affected by land use and rainfall characteristics
Project Number:	2012KY204B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 6th
Research Category:	Ground-water Flow and Transport
Focus Category:	Hydrology, Groundwater, Solute Transport
Descriptors:	infiltration, soil properties
Principal Investigators:	Ole Wendroth

Publication

1. Yang, Yang, Ole Wendroth, and Riley Walton, 2013, Field-Scale Bromide Leaching as Affected by Land Use and Rainfall Characteristics, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 35

Field-Scale Water and Bromide Transport as Affected by Land Use and Rainfall Characteristics

Problem and Research Objectives

A precise description of water and solute transport through the soil is required for predicting the efficiency of nutrient amendment and its impact on environmental quality (Feyen et al., 1998; Paramasivam et al., 2002). Many investigations have been conducted on soil columns under laboratory conditions; while at the field scale, little progress has been made due to natural soil heterogeneity (Nielsen et al., 1986; Ashraf et al., 1997) and the lack of an effective methodology for quantifying solute flux under transient flow conditions. A field technique that combines water flow and solute transport to quantitatively determine the fate of surface-applied solutes is needed. This is especially true in the well- drained soils of Kentucky, where rapid water infiltration can cause deep leaching of surface-applied chemicals.

The main objective of this project was to quantify the influence of rainfall characteristics on water and bromide transport within and across two common land use systems in Kentucky (cropland and grassland) to answer the following questions:

- 1) How do the amount and intensity of rainfall and land use affect the leaching behavior of a surface applied salt?
- 2) How does the time between surface application of bromide and subsequent rainfall affect the leaching behavior of bromide?
- 3) Can a treatment effect on bromide leaching be identified under field conditions?
- 4) Can water and solute transport processes be efficiently modeled under field conditions?

Methodology

The experiment was conducted at Spindletop Research Farm, Lexington, KY. The region receives an average annual precipitation of 114 cm and the mean annual temperature is 13 °C. From April through September, mean annual potential evapotranspiration exceeds rainfall. A transect, evenly distributed across two established land use systems (cropland and grassland) was divided into plots to investigate the transport of water and bromide (Br^-) under different rainfall conditions. Three levels of rainfall intensity (25, 50, 75 mm/hr), and four levels of time delay between solute application and subsequent rainfall (1, 6, 24, 96 hr) were arranged in a repetitive pattern at different scales along the transect. Soil samples in 10 cm increments were collected down to 1 m depth along the transect for Br^- analysis after rainfall simulations.

Principal Findings and Significance

Soil Br^- was more evenly distributed with soil depth and reached greater depth in the grassland treatment, likely due to more continuous macropores creating preferential flow paths. Increasing rainfall intensity tended to enhance the deep leaching of Br^- . Frequency-domain analysis revealed that the dominant factor that controlled Br^- leaching varied with depth. At 0-10 cm, rainfall intensity was most strongly correlated with Br^- concentration. In the layer right below, application time delay was more closely linked to the spatial distribution of Br^- . With

increased soil depth, the spatial behavior of Br^- was mainly related to soil properties such as texture and surface topography rather than rainfall characteristics. Nevertheless, rainfall intensity was found to be positively correlated with Br^- concentration in deep soil horizons, indicating a great risk of deep leaching and groundwater contamination under heavy rainfall. Results of the study suggest that this experimental design is useful in studying hydrological processes at field scale.

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Fate & transport of herbicides in karst autogenic recharge of Kentucky's Mississippian Plateau

Basic Information

Title:	Fate & transport of herbicides in karst autogenic recharge of Kentucky's Mississippian Plateau
Project Number:	2012KY205B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 2nd
Research Category:	Ground-water Flow and Transport
Focus Category:	Solute Transport, Groundwater, Non Point Pollution
Descriptors:	atrazine, infiltration, vadose zone, epikarst
Principal Investigators:	Chris Groves

Publication

1. Groves, Chris, Robert Lerch, Jason Polk, Ben Miller, and Sean Vanderhoff, 2013, Herbicide Transport Within Shallow Karst Groundwater on Kentucky's Pennyroyal Plateau Beneath Row Crop Agriculture, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 5-6

Fate & Transport of Herbicides in Karst Autogenic Recharge on Kentucky's Mississippian Plateau

Problem and Research Objectives

More than half of Kentucky is underlain by relatively soluble carbonate rock, primarily limestone. Dissolution has resulted in karst landscape/aquifer systems in which caves, underground rivers, and large springs are common. These features are well developed over at least 25% of the state (Currens, 2002). Because of the typically high permeability of these aquifers, recharge and contaminants that may be present in it can infiltrate quickly and move rapidly once underground. These groundwater systems are often extremely vulnerable. This can threaten drinking water sources and create adverse ecological impacts (including on federally endangered species) as karst aquifers have relatively high ecosystem diversity compared to other groundwater systems. The best-developed and most widespread karst in Kentucky is on the Pennyroyal Plateau, a region that also has extensive agriculture. Potential pollutants including fecal bacteria, pesticides, and nutrients are widespread and details about the fate and transport of these contaminants and relevant hydrologic and biogeochemical behaviors, and how they may be related to specific land use practices are incompletely described.

The purpose of this research project was to clarify the timing and spatial characteristics of infiltration, storage, and transport of herbicides following real-world spring application within the Graham Springs Groundwater Basin (Ray and Currens, 1998, 2000), a well developed karst aquifer/landscape of the Pennyroyal Plateau. The field site is Crumps Cave in northern Warren County, Kentucky, a large horizontal cave that passes under active row crop and cattle farming, with several areas in the cave that have perennial waterfalls. These have formed as subsurface drains beneath the shallow water storage of the epikarstic zone in the vicinity of the soil bedrock interface. Through tracing experiments, a connection has been made between a roughly one hectare (Polk *et al.* 2010) section of a field undergoing active row crop farming and an epikarst drain (Waterfall 1—WF1) in the cave about 150 m laterally and 25 m below the field. WF1 represents the point at which water leaves epikarst storage and enters the main part of the karst aquifer. At WF1 it is possible to monitor the movement and chemistry of *autogenic* recharge infiltrating as rainfall lands on a specific field with homogeneous land use. This is recharge that lands directly on and infiltrates as diffuse flow into the soil lying directly above the karst aquifer (White, 1988; Ford and Williams, 2007), in contrast to *allogenic* recharge that enters the aquifer system at discrete locations such as sinking streams. Much of the recharge into the karst systems of Pennyroyal Plateau impacted by agricultural contamination is of this type, and it is particularly difficult to isolate the impact of a particular land use because many springs in the region at which water quality can be monitored have catchments of tens to hundreds of square kilometers within which a variety of land use impacts are simultaneously present.

After passing through the soil and the epikarstic zone and flowing over WF1, the water continues laterally through the main part of the karst aquifer to resurge at Wilkins Bluehole on the Barren River, 18 km to the southwest. Wilkins Bluehole is the second largest spring in Kentucky, with a minimum discharge of 0.56 m³/s (Ray and Blair 2005; Ray and Currens 1998, 2000). With an automated weather station and water collection on the surface, lysimeters in the

soil, and the ability to monitor in the cave below the epikarstic zone and at Wilkins Blue Hole, it is possible to track the timing and spatial distribution of tracers passing through this field, either those introduced by farming or inputs added as controlled experiments.

In this project we monitored the behavior of herbicides introduced through row crop farming in the field intermittently for parts of two years after introduction of a product containing the herbicide atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) at the site in spring 2011. Funding from this grant supported fieldwork and analysis during 2012 and 2013 using an ELISA immunoassay screening procedure, as well as additional higher-resolution analysis with gas chromatography–mass spectrometry (GC-MS) for several different herbicides and their principal metabolites, including the 2011 samples that had been previously preserved by freezing.

Methodology

The experimental field and Crumps Cave below are located in northern Warren County, Kentucky. The site is underlain by Crider silt loam, Pembroke silt loam and Baxter gravelly silt loam soils (Soil Survey Staff NRCS, 2011). The cave is located in the upper part of the Mississippian St. Louis Limestone, with local dip of about 1-2° towards the west (Richards, 1964). At the surface, we collected rainfall and other atmospheric data with ten-minute resolution. At WF1, about 50 m from the cave's entrance (which is gated and thus safe from equipment vandalism), we collected continuous discharge by directing the flow into a barrel-shaped weir, and checking weekly against discharge directly measured by timing the flow of a fixed volume. Data loggers collected water level in the weir, pH (triplicate), specific conductance and temperature (duplicate) with ten-minute resolution (Groves *et al.* 2005; Bolster *et al.* 2006). Two automated water samplers were also installed at the site.

At the site, we monitored farming activity to establish timing of treatments including application of manure and herbicides. We began the project in January 2011 (before current funding was available) using student time supported by other resources. Samples were taken on varying schedules, primarily during storm events. Many of these samples were preserved by freezing and saved for later analysis. Samples were analyzed in our laboratory using enzyme-linked immunosorbent assay (ELISA) and a subset of those samples were analyzed using gas chromatography–mass spectrometry (GC-MS) using the methods of Lerch and Blanchard (2003) at the USDA Agricultural Research Service laboratory in Columbia, Missouri. ELISA (detection limit ~0.5 µg/L) was utilized for the principal herbicide, and GC-MS provided information to check calibration of the ELISA samples and data on a variety of compounds (atrazine, simazine, acetochlor, alachlor, metolochlor, and the atrazine metabolites deethylatrazine (DEA) and deisopropylatrazine (DIA)).

A significant change in the methodology from our proposal occurred because no herbicides were applied to the 2012 crops chosen by the farmer. Although the site is physically appropriate for studying the impacts of row crop agriculture on epikarst water, we were not able to control the treatments in a given growing season. As a consequence, we were not able to track the transport of a new slug of chemical as we had proposed to do, but we were able to get additional relevant data from the 2011 slug through additional GC-MS analysis of preserved

samples, especially the metabolites and their ratios, and continue monitoring the site to learn about the persistence of these chemicals in the soil/epikarstic zone through a second year following application.



Figure 1. Epikarst water monitoring site in Crumps Cave.

We continued sampling through the end of May 2013. Here were report on the 2011 and 2012 results.

Principal Findings and Significance

Prior to the spring 2011 herbicide application, we found low but persistent levels of atrazine and the metabolites DIA and DEA, nearly all positive hits but typically less than 1 $\mu\text{g/l}$. Herbicides were applied in early March 2011 to a field about 150 m laterally and 25 m above the in-cave monitoring site. There was a two-month delay for first major pulse of atrazine, which had levels up to 38 $\mu\text{g/l}$ (more than 12 times the Environmental Protection Agency's maximum contaminant level (MCL) for drinking water), to come through the system. This arrived during a storm event, but followed several other storm responses that previously came through the epikarst system without elevated levels of herbicide. Several less concentrated pulses came through in subsequent storms, and eventually continuous, sustained levels typically between 0.1 and 1 $\mu\text{g/l}$ were maintained over all subsequent sampling.

Important questions arise: where was the atrazine during the two months it initially took to get to the cave and subsequently why did it continue leaking at low levels into the system? The metabolite ratio data (Figure 2) tells a compelling story. We computed the dealkylated metabolite to parent ratio (DMPR) and the DEA to atrazine ratio (DAR) because DIA was generally very significant in these samples. Both ratios reflect high levels of metabolite to parent during the winter, a precipitous drop in the ratio with new atrazine transport in early May and then a near linear increase through the summer and early fall. Both sites then show some additional transport in the fall with proportionally more atrazine and a commensurate decrease in the ratios. The summer ratios at WF1 reached nearly twice that of ratios of the previous winter pre-application period. This shows that the metabolites dominate in transport through the course of the summer and early fall. All of this supports a hypothesis of atrazine slowly leaching through the soil column such that significant degradation occurred, as opposed to fast atrazine transport to the epikarst aquifer and subsequent storage before eventual breakthrough to the cave. In other words, breakthrough suggests that most of the atrazine stayed in the biologically active soil zone and degraded through the course of the year. The parent that did make through the system was simply a small portion of the applied atrazine that was not subject to microbial breakdown. It must have been transported deeper into the soil and slowly moved through the sub-soil before reaching the epikarst and eventually the cave. The delayed transport likely indicates that the atrazine was slowly moving through the soil column on its way to the cave.

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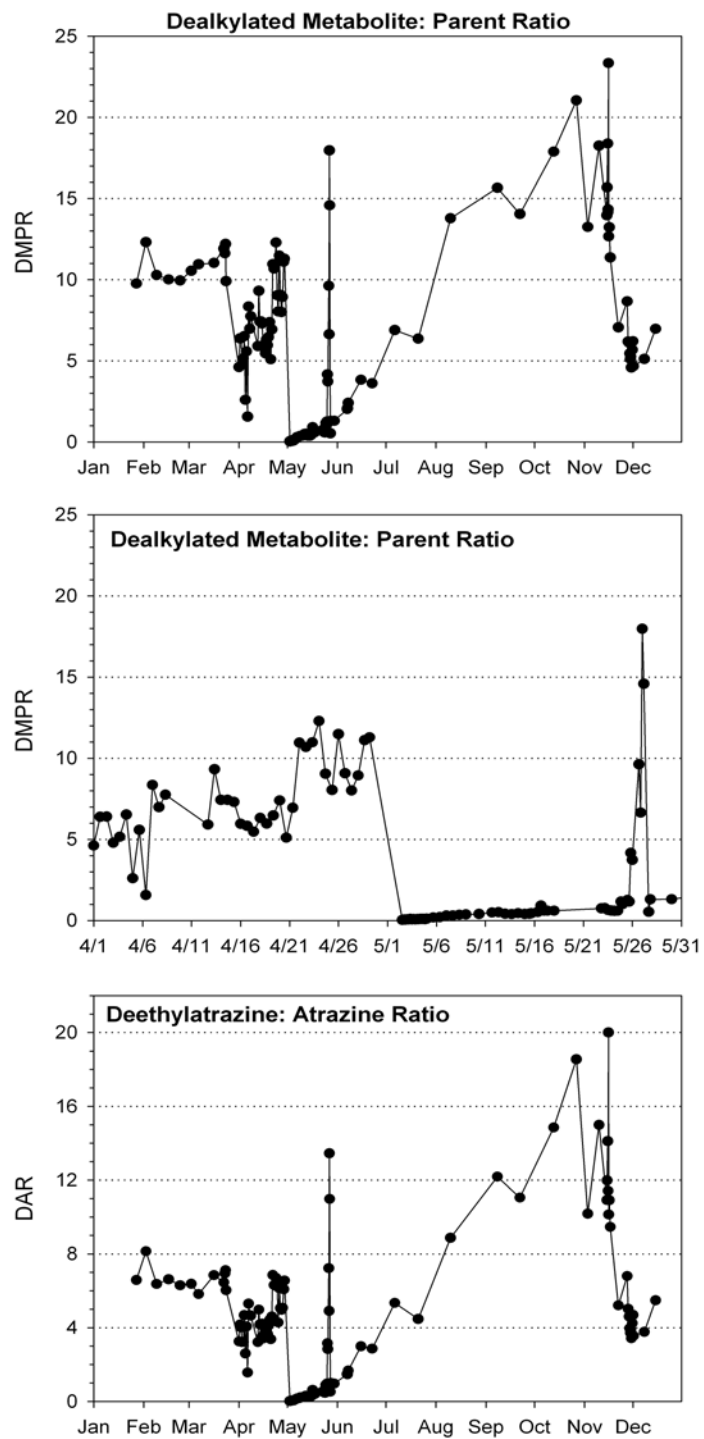


Figure 2. Parent-metabolite ratios that help establish storage conditions of atrazine in the soil and epikarst.

Arsenic detection using quartz crystal microbalance

Basic Information

Title:	Arsenic detection using quartz crystal microbalance
Project Number:	2012KY206B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 6th
Research Category:	Water Quality
Focus Category:	Water Quality, Methods, Toxic Substances
Descriptors:	selenite, receptor ligand, dithiol ligand
Principal Investigators:	David A. Atwood

Publication

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Arsenic Detection Using Quartz Crystal Microbalance

Problem and Research Objectives

Arsenic is a well-known contaminant of drinking water that poses severe adverse health risks across the globe. Most of the current methods for the detection of selenium and arsenic require pre-concentration or digestion prior to analysis on a laboratory instrument such as inductively coupled plasma mass spectrometry. Current methods consequently do not allow onsite detection and can have long turnaround times for analysis results. This study evaluated optimization of conditions for real time selenium and arsenic detection with very low limits of detection using a quartz crystal microbalance. The overall goal of the project was to develop a fully functional mobile arsenic detection sensor that will be low cost, user friendly, and able to be monitored in remote locations.

Methodology

In the quartz crystal microbalance, a quartz crystal oscillates at its resonance frequency and any changes in mass on the crystal cause a change in the resonant frequency which can be measured and related back to additional mass. The sensitivity of this detection method can measure changes in mass as small as 100 ng [1]. Attaching a ligand to the surface of a quartz crystal should allow for detection of metal and metalloid ions in solution based on their interaction with the receptor ligand. Figure 1 shows the structure of a receptor that can be attached to the quartz crystal. It is a carboxylic acid derivative (abbreviated AB9) of the parent compound N, N'- bis(2-mercaptoethyl)isophthalamide (abbreviated B9). B9 and AB9 have been shown to remove arsenic from water and bonding to the ligands has been established. This work focused on investigating the reaction mechanism and final products of the reaction of the dithiol ligand B9 with selenite. Small alkylthiol compounds undergo a series of reaction steps, including formation of alkylthioselenic acid, bis(alkylthio)selenoxide, sulfenic acid, and thiosulfonate before eventually producing elemental selenium and disulfide [2]. Based on pH and other factors, the reaction can be stopped at various points along the reaction steps.

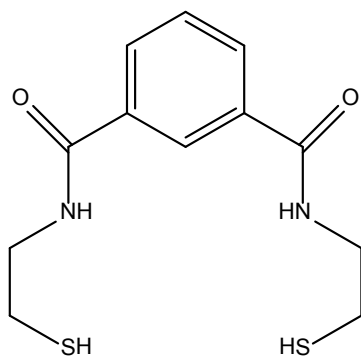
Principal Findings and Significance

Reaction products of selenite with the dithiol ligand were characterized using infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectrometry, differential scanning calorimetry, and thermo gravimetric analysis.

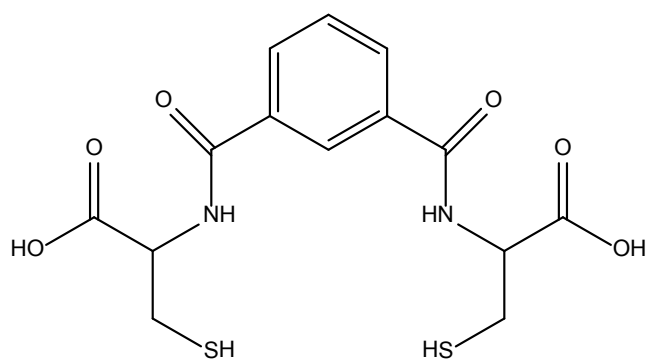
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Figure 1: Structure of B9 and AB9:



B9



AB9

Introduction

Most of the current methods for arsenic and selenium detection are not capable of real-time, on-site detection. The turnaround time for such methods can be rather long. This research project investigated arsenic and selenium detection using a receptor molecule covalently bonded to the surface of a quartz crystal microbalance (QCM).

The compound used for the receptor is a carboxylic acid derivative (AB9) of the parent compound N,N'-bis(2-mercaptoethyl)isophthalamide (abbreviated B9) (Fig 1). The carboxylic acid groups of AB9 allow it to be attached the surface of the quartz crystal by formation of an amide bond with an organic linker on the surface. Most of the metals and metalloids previously studied by our group are covalently bound by the ligands.^{1,2} When the ligand is attached to the quartz crystal (Fig. 2) the formation of the covalent bond with the metal or metalloid produces a small mass change, which causes a measurable change in the resonant frequency of the crystal, from which mass change can be calculated. Once a preliminary test demonstrated that arsenite could be detected in real-time with the Receptor-QCM combination, the research focused on obtaining fundamental chemical information about the receptor-analyte bonding.

Figure 1. B9 and AB9

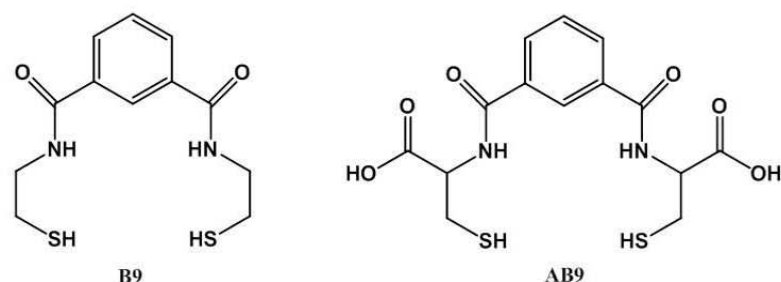
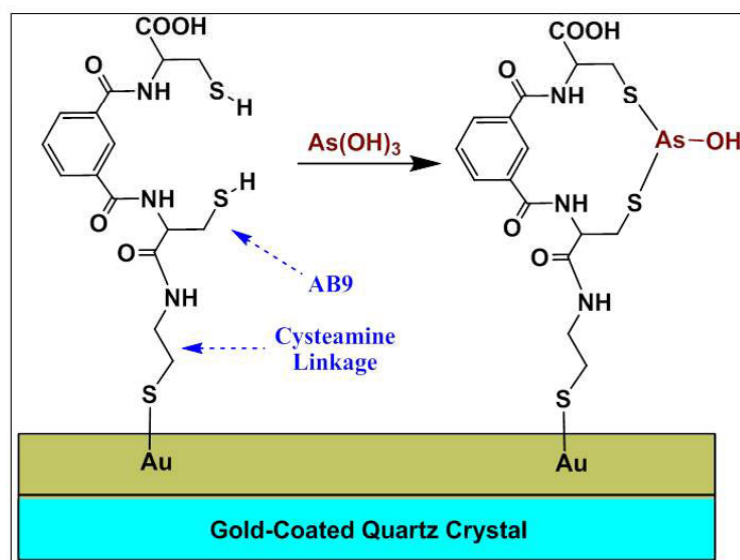


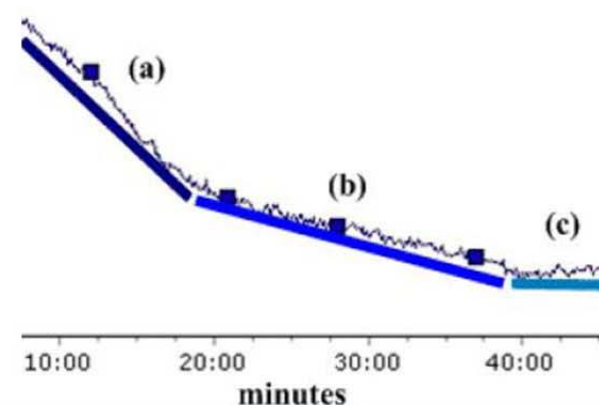
Figure 2. QCM Crystal With AB9 for As(III) Detection



Arsenic Detection

A preliminary experiment demonstrated arsenic detection using QCM. Figure 3 shows the QCM response when an As(III) solution is passed over the quartz crystal. There is initially a steady decrease in frequency due to As(III) binding, but eventually the frequency does not decrease due to saturation of the ligand binding sites.

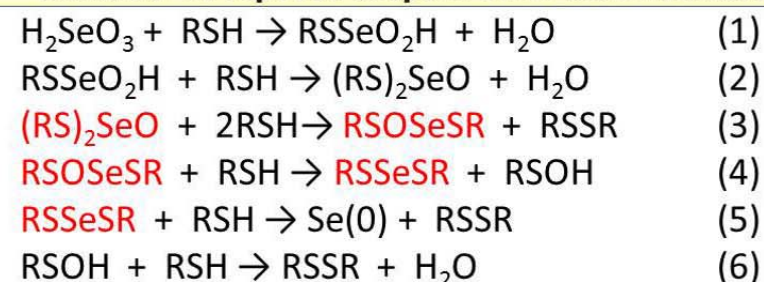
Figure 3. QCM Graph After Injection of 200ppb As(III)



Selenium Interaction with Ligand

When selenite, Se(IV), is introduced to B9 at ambient pH it undergoes a series of reactions rather than formation of a stable covalent bond. Scheme 1 shows a proposed series of reaction steps in the reaction of thiols with selenite that ultimately results in formation of elemental selenium and disulfide.³

Scheme 1. Proposed Steps of Thiol-Selenite Reaction



NMR Study of B9-Se Reaction Intermediate

The B9-Se reaction intermediate was isolated by conducting the reaction in acidic conditions. The product was only soluble in dimethyl sulfoxide. Due to lack of solubility, NMR was the best characterization available. Figure 4 shows the possible structures of the product. The ¹H-NMR spectrum contained too many overlapping peaks to make any peak assignments. The ¹³C-NMR spectrum indicates a symmetrical molecule, ruling out the possibility of structure Fig. 4c. The ⁷⁷Se-NMR spectrum (Fig. 5) has two peaks in the range appropriate for divalent Se species, ruling out the possibility of the structure shown in Fig. 4b. The presence of two peaks is attributed to BDT-Se (major) and Se(0) (minor). From the NMR data it was determined that the structure was the symmetrical, divalent structure shown in Fig. 4a.

Figure 4. Possible Structures of B9-Se

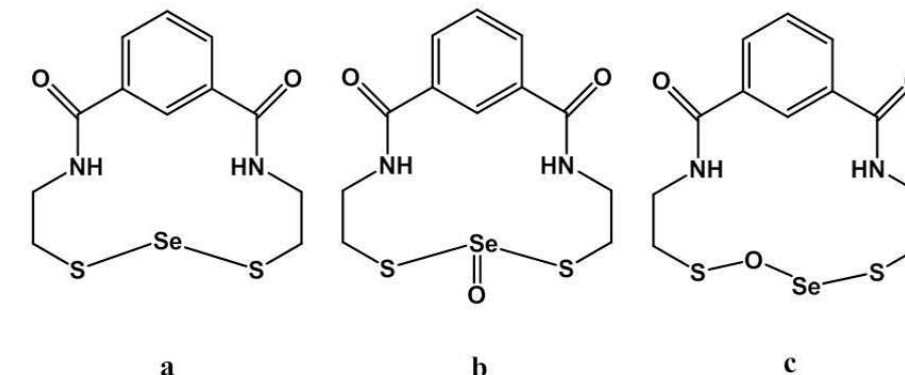
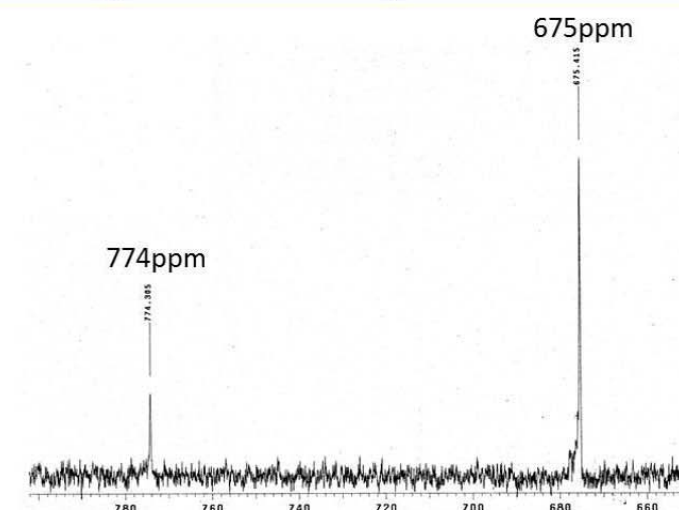


Figure 5. ⁷⁷Se-NMR Spectrum of B9-Se⁴



Conclusions

- Arsenite detection using QCM has been demonstrated
- B9-Se product determined to be structure shown in Figure 4a
- New electronic designs have been investigated for the QUANSOR⁵ QCM

Future Research

- Determine interferences for As and Se detection
- Determine if ligand can be regenerated after As and Se detection
- Detection in "challenge waters" with naturally-occurring cations and anions

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- John Layton at University of Kentucky NMR Center for NMR assistance
- This work was done in collaboration with QUANSOR Inc.

Sampling for pharmaceuticals in Kentucky surface waters

Basic Information

Title:	Sampling for pharmaceuticals in Kentucky surface waters
Project Number:	2012KY207B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 1, 2, and 5
Research Category:	Water Quality
Focus Category:	Water Quality, Toxic Substances, Surface Water
Descriptors:	estradiol, fluoroquinolones
Principal Investigators:	E. Glynn Glynn Beck

Publication

1. Beck, E.G., R. DeJaco, S. Webb, A. Fogle, D. Williams, M. Silitonga, and B. Lee, 2013, The Presence of 17-B Estradiol and Fluoroquinolones in Kentucky's Surface Water, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 5-6

Sampling for Pharmaceuticals in Kentucky Surface Waters

Problem and Research Objectives

Over the past decade, surface water sampling conducted by the U.S. Geological Survey (USGS) and others (Hedgespeth and others, 2012) has shown the presence of pharmaceuticals and personal care products (PPCPs) in the environment. In 1999 and 2000, the USGS sampled 139 streams in 30 states for 95 pharmaceutical and personal care product chemicals. One or more of the chemicals analyzed were found in 80 percent of the streams sampled (Barnes and others, 2002; Buxton and Kolpin, 2002; Kolpin and others, 2002a; Kolpin and others, 2002b). In 2008, the USGS, in cooperation with the Kentucky Division of Water (KDOW), sampled nine selected watersheds in Kentucky for PPCPs. Surface water samples analyzed from seven of the nine watersheds contained one or more pharmaceuticals and antibiotics (Angie Crain, USGS Kentucky Water Science Center, personal communication).

Prior to receiving funding for this USGS 104b project, Kentucky was one of 10 states involved in a USDA-NIFA Southern Regional Water Program pilot project to sample surface water for 17- β Estradiol and fluoroquinolones. As part of the USDA-NIFA Southern Regional Water Program project, 19 sites within the Floyds Fork hydrologic unit code (HUC) 11-digit watershed were sampled for 17- β Estradiol and fluoroquinolones.

17- β Estradiol, or estradiol, is a hormone steroid naturally produced by humans and animals. Studies conducted in Europe (Larsson and others, 1999), the United States (Kolpin and others, 2002a) and Canada (Servos and others, 2005) have shown that estradiol is present in surface water. Estradiol is a known endocrine disruptor, which may have a deleterious effect on aquatic wildlife (Purdom and others, 1994; Jobling and others, 1998). Potential sources of estradiol to surface water are effluent from waste water treatment plant (WWTP) outfalls, surface runoff from animal manure amended fields, and effluent from domestic straight pipes.

Fluoroquinolones are a group of broad spectrum antibacterials used to treat humans and animals (Andersson and MacGowan, 2003; McEwen and Fedorka-Cray, 2002). Like estradiol, studies in Europe (Andreozzi and others, 2003) and the United States (Kolpin and others, 2002a) have shown that fluoroquinolones are present in surface water. In 2005, The U.S. Food and Drug Administration banned the use of fluoroquinolones in poultry production (Nelson and others, 2007). The ban was implemented because of the development of human infections with fluoroquinolone-resistant *Campylobacter* species associated with the consumption of poultry (Nelson and others, 2007). Currently, the effects of fluoroquinolones, if any, on aquatic wildlife are unknown. Potential sources of fluoroquinolones to surface water are the same as those of estradiol.

The objectives of this USGS 104b project were to:

- 1) Expand surface-water sampling for the pharmaceuticals estradiol and fluoroquinolones to six additional HUC 11-digit watersheds distributed across Kentucky,
- 2) Build on existing/ongoing emerging contaminant research in Kentucky, and
- 3) Train an undergraduate student in field sampling and laboratory analytical methods.

Because of changes in the number of samples collected in each watershed, only five HUC 11-digit watersheds were sampled instead of six as described in objective one.

Methodology

The five sampled watersheds were chosen to represent different physiographic regions of Kentucky and different land uses. Two of the watersheds are located in western Kentucky (Bayou de Chien and Clarks River), one in central Kentucky (South Elkhorn Creek), one in northern Kentucky (Banklick Creek), and one in eastern Kentucky (Licking River) (Fig. 1). Four of the five watersheds contain at least one mapped municipal WWTP outfall (Table 1). Land cover for each watershed is listed in Table 1. Also, four (Clarks River, South Elkhorn Creek, Banklick Creek, and Licking River) of the five watersheds are classified by the KDOW as priority watersheds because of various impairments.



Figure 1. Map showing the location of the Bayou de Chien, Clarks River, South Elkhorn Creek, Banklick Creek, and Licking River watersheds.

Table 1. Land-cover type (Homer and others, 2007) and number of municipal WWTP outfalls in each watershed.

Watershed	Land Cover (percent)					Number of WWTP Outfalls
	Forest	Pasture	Cultivated	Developed	Other	
Bayou de Chien	14	9	58	5	14	1
Clarks River	32	13	42	9	4	5
South Elkhorn Creek	8	63	4	24	1	2
Banklick Creek	36	24	1	36	3	0
Licking River	82	12	1	4	1	1

Google™ Earth was used to locate sampling sites within each watershed that corresponded with highway bridges or WWTP property adjacent to the stream sampling site. Table 2 lists the number of sampling sites per watershed. In June 2012, during extremely low flow conditions, each site was sampled following methods described by Wilde and others (2004). One 40 ml grab sample was collected in an amber glass vial from each site. In order to eliminate potential sample contamination, vials were submerged in the stream approximately four inches before the cap was removed. While submerged the vial cap was removed allowing the vial to fill with water and the cap was then screwed tightly onto the vial. To ensure uniform mixing, if possible, samples were collected in flowing water. Also, if possible, samples collected downstream of WWTP outfalls were collected far enough away from the outfall to ensure uniform mixing of treated raw water and stream water. Sample bottles were placed on ice and kept out of direct sunlight until submitted to the Kentucky Geological Survey Laboratory Services. All samples were delivered to the Kentucky Geological Survey Laboratory Services within 24 hours of collection. Fluoroquinolones splits were made for each sample by laboratory personnel and preserved with 10 percent methanol within 24 hours of collection. Abraxis LLC Enzyme-linked immunosorbent assay (ELISA) kits were used in conjunction with a ChroMate® 4300 Microplate Reader to analyze all samples. Analytical methods were followed as described in ELISA kit instructions. All estradiol and fluoroquinolones samples were analyzed in the Kentucky Geological Survey Laboratory Services within 7 and 14 days of collection, respectively. Two reps of each analyte were analyzed per sample. At least one duplicate sample was collected from each of the watersheds (Table 2). Blank samples (laboratory deionized water) were analyzed for 4 of the 5 watersheds (Table 2). Blank sample vials were filled in the field and were exposed to the same storage and preservation methods as the field samples.

Method detection limits (MDL) for estradiol and fluoroquinolones were calculated by Kentucky Geological Survey Laboratory Services personnel using U.S. EPA procedures (U.S. EPA, 2011). The MDL for estradiol and fluoroquinolones is 3.0 parts per trillion (ppt) and 0.025 parts per billion (ppb), respectively.

A Horiba U-22 water-quality meter was used to collect field measurements of pH, specific conductance, temperature, dissolved oxygen, Eh, and TDS. The Horiba U-22 was calibrated daily according to manufacturer's instructions. Stream discharges were measured, when possible, at each sampling site, that did not have an operating USGS stream gage station, at the time of sample collection using a Marsh-McBirney flow meter and the six-tenths-depth

method as described in Buchanan and Somers (1976) (Fig. 2). When possible, stream discharge measurements were obtained on-line from USGS stream gage stations [http://ky.water.usgs.gov/hyd_data/gagemap_2012_links.pdf]. USGS discharge data were used to identify the relative flow conditions under which the stream samples were collected.

Sample sites were geo-referenced using a handheld Magellan eXplorist 200 GPS unit. Detailed information related to sample locations is listed in Table 3.

Table 2. The number of sampling sites and duplicate and blank samples collected per watershed.

Watershed	Number of Sampling Sites	Number of Duplicate Samples	Number of Blank Samples
Bayou de Chien	4	1	0
Clarks River	6	1	1
South Elkhorn Creek	11	2	1
Banklick Creek	6	1	1
Licking River	10	2	1
Total	37	7	4

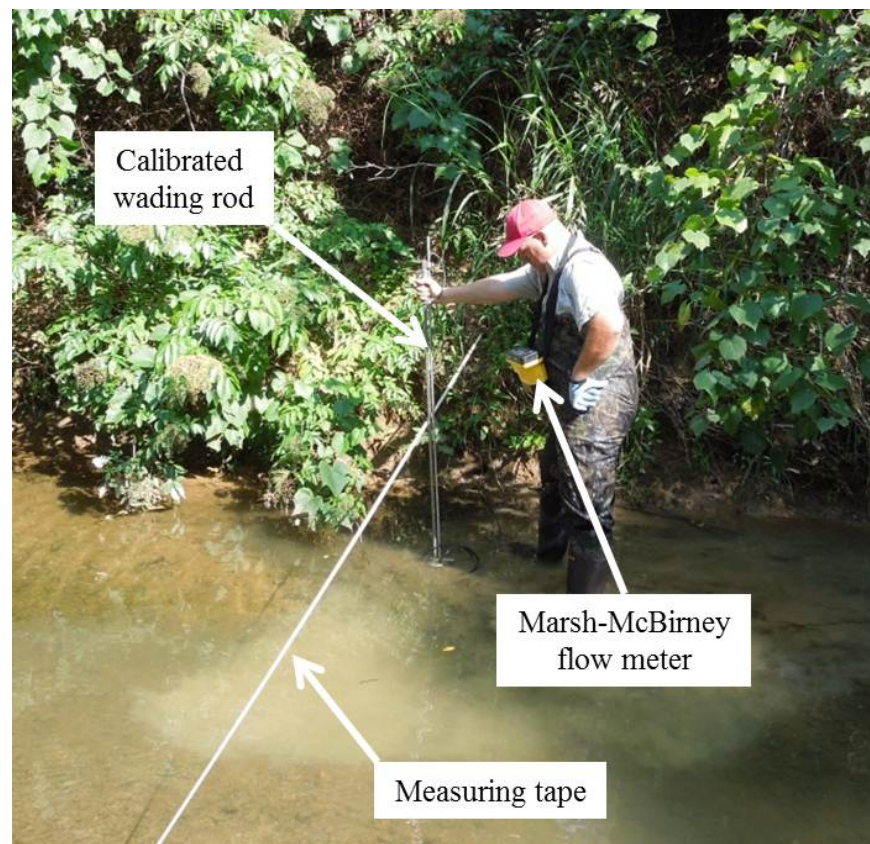


Figure 2. Equipment and technique used to measure in-stream discharge.

Table 3. Location information for samples collected in Bayou de Chien (BdC), Clarks River (CR), South Elkhorn Creek (SEC), Banklick Creek (BC), and Licking River (LR) watersheds.

Site Name	Stream Name	County	Latitude	Longitude	Sample Collection Site	Sample Collected Upstream or Downstream of Outfall
BdC 1	Bayou de Chien	Hickman	36.604233	-88.816826	In stream	
BdC 2	Bayou de Chien	Hickman	36.628544	-88.964501	In stream	
BdC 3	Little Bayou de Chien	Fulton	36.595713	-89.033837	Bridge	
BdC 4	Bayou de Chien	Fulton	36.584717	-89.169357	Bridge	Downstream
CR 1	Middle Fork Clarks River	Calloway	36.578050	-88.388440	In stream	
CR 2	Bee Creek	Calloway	36.629135	-88.294774	In stream	Upstream
CR 3	Bee Creek	Calloway	36.630341	-88.291547	In stream	Downstream
CR 4	Clarks River	Calloway	36.691647	-88.273533	In stream	
CR 5	Clarks River	Marshall	36.866796	-88.331376	In stream	
CR 6	Clarks River	Marshall	36.873453	-88.346467	In stream	
SEC 1	Town Branch	Fayette	38.060054	-84.529865	In stream	Upstream
SEC 2	Town Branch	Fayette	38.063772	-84.534635	In stream	Downstream
SEC 3	Wolf Run	Fayette	38.067057	-84.554304	In stream	
SEC 4	Town Branch	Fayette	38.103799	-84.587987	In stream	
SEC 5	South Elkhorn Creek	Fayette	38.025861	-84.617978	In stream	
SEC 6	Shannon Run	Fayette	38.043164	-84.649086	In stream	
SEC 7	South Elkhorn Creek	Woodford	38.103264	-84.641147	In stream	
SEC 8	South Elkhorn Creek	Scott	38.141297	-84.645067	In stream	
SEC 9	Lee Branch	Woodford	38.162232	-84.686456	In stream	Upstream
SEC 10	Lee Branch	Woodford	38.162555	-84.686487	In stream	Downstream
SEC 11	South Elkhorn Creek	Franklin	38.213588	-84.799480	In stream	
BC 1	Brushy Fork	Kenton	38.953681	-84.553403	In stream	
BC 2	Banklick Creek	Kenton	38.962082	-84.556567	Bridge	
BC 3	Fowler Creek	Kenton	38.979778	-84.540640	In stream	
BC 4	Banklick Creek	Kenton	38.980442	-84.541860	In stream	
BC 5	Banklick Creek	Kenton	38.996486	-84.538850	In stream	
BC 6	Banklick Creek	Kenton	39.034747	-84.530595	In stream	
LR 1	Licking River	Magoffin	37.599980	-82.959940	In stream	
LR 2	Trace Fork	Magoffin	37.600536	-82.960970	In stream	
LR 3	Puncheon Camp Creek	Magoffin	37.647162	-82.999182	In stream	
LR 4	Big Half Mount Creek	Magoffin	37.651191	-83.013566	In stream	
LR 5	Oakley Creek	Magoffin	37.678402	-83.048085	In stream	
LR 6	Licking River	Magoffin	37.679438	-83.048324	In stream	
LR 7	Licking River	Magoffin	37.731944	-83.060752	In stream	
LR 8	Licking River	Magoffin	37.749184	-83.093415	In stream	Upstream
LR 9	Licking River	Magoffin	37.748972	-83.093670	In stream	Downstream
LR 10	Licking River	Magoffin	37.783589	-83.143661	In stream	

Principal Findings and Significance

Bayou de Chien Watershed

The Bayou de Chien watershed is located in southwestern Graves, southern Hickman, and most of Fulton Counties (Fig. 1). Bayou de Chien begins in southwestern Graves County and flows to the west toward the Mississippi River. The dominate land cover in the watershed is cultivated (Table 1; Fig. 3). There are approximately 125 poultry houses located within the watershed and most of the associated litter is amended to row crop fields within the watershed. The City of Hickman's WWTP outfall, located east of sample site 4, is the only mapped WWTP outfall in the watershed (Fig. 3). The population of Hickman is approximately 2,400 people.

Four sites were sampled in the watershed (Fig. 3). Because of steep stream banks at sites 3 and 4, collecting in-stream samples was not possible. Therefore, sites 3 and 4 were sampled from a highway bridge (Table 3). Field measurement data collected at two of the sites (sites 1 and 2) are shown in Table 4. Field measurements were not taken at sites 3 and 4 because of the inability to take in-stream measurements.

Discharge measurements were only obtained from two of the sites (sites 1 and 2). In-stream discharge was measured at site 1 using a Marsh-McBirney flow meter (Table 5). A discharge measurement for site 2 was obtained from a USGS stream gage station installed at site 2 (Table 5). Because of the inability to access the stream, discharge measurements were not taken at sites 3 and 4. On the day of sampling, June 25, 2012, the discharge at site 2 was 18.00 cubic feet per second (cfs) (Table 5). As depicted in Figure 4, Bayou de Chien watershed samples were collected during low flow conditions.

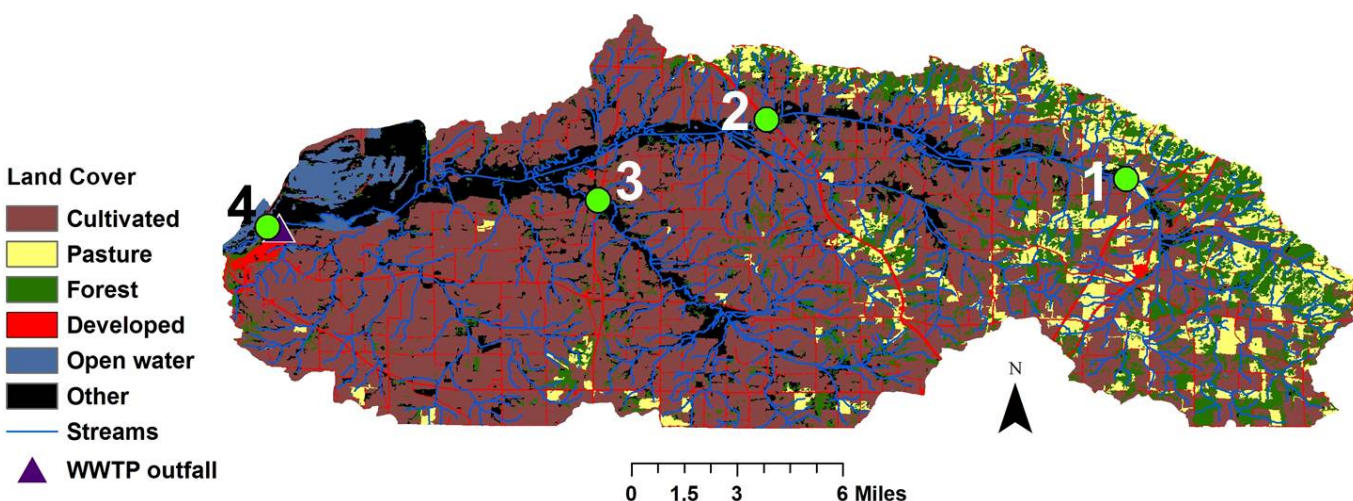


Figure 3. Map of the Bayou de Chien watershed with land cover (Homer and others, 2007). Green circles are sampling site locations with corresponding sample number.

Table 4. Field measurements collected at each sampling site in the Bayou de Chien (BdC), Clarks River (CR), South Elkhorn Creek (SEC), Banklick Creek (BC), and Licking River (LR) watersheds. A blank cell indicates that a field measurement was not recorded.

Site Name	Date	Time	Field pH	Field Specific Conductance (mS/cm)	Field Temperature (°C)	Field Dissolved Oxygen (mg/L)	Field Eh (mV)	Field TDS (mg/L)
BdC 1	6/25/2012	14:25	6.66	0.062	23.95	9.32	189	40
BdC 2	6/25/2012	15:22	6.91	0.074	27.70	8.60	170	50
BdC 3	6/25/2012	15:56						
BdC 4	6/25/2012	16:30						
CR 1	6/25/2012	8:12	6.55	0.101	23.38	5.17	202	70
CR 2	6/25/2012	9:09	6.82	0.276	22.81	5.74	251	180
CR 3	6/25/2012	9:39	7.25	0.515	22.61	8.52	205	330
CR 4	6/25/2012	10:38	7.22	0.322	26.55	7.28	207	210
CR 5	6/25/2012	11:31	6.95	0.172	25.75	6.79	210	110
CR 6	6/25/2012	12:24	6.87	0.172	27.65	5.70	248	110
SEC 1	6/19/2012	8:19	7.25	0.883	19.69	6.67	129	570
SEC 2	6/19/2012	9:18	7.26	0.812	20.56	8.62	144	520
SEC 3	6/19/2012	10:10	7.54	0.767	21.25	6.89	137	490
SEC 4	6/19/2012	10:33	7.41	0.732	22.78	7.30	166	470
SEC 5	6/19/2012	11:09	7.57	0.549	25.07	5.94	157	350
SEC 6	6/19/2012	11:42	7.42	0.334	28.55	5.03	162	220
SEC 7	6/19/2012	13:09	7.39	0.430	28.84	4.36	188	280
SEC 8	6/19/2012	14:02	7.57	0.699	26.08	6.77	158	450
SEC 9	6/19/2012	14:30	7.75	0.461	25.43	6.71	161	300
SEC 10	6/19/2012	15:00	7.55	0.487	25.75	6.67	184	320
SEC 11	6/19/2012	15:38	7.95	0.550	26.99	8.81	175	350
BC 1	6/27/2012	10:35	7.12	0.595	23.75	4.27	218	380
BC 2	6/27/2012	10:54						
BC 3	6/27/2012	11:26	8.15	0.834	20.38	8.79	189	530
BC 4	6/27/2012	11:59	8.08	0.670	22.95	7.92	200	430
BC 5	6/27/2012	13:32	8.28	0.628	25.55	8.79	201	400
BC 6	6/27/2012	14:07	8.24	0.736	26.26	9.22	199	470
LR 1	6/20/2012	12:28	7.47	1.210	24.82	7.07	151	800
LR 2	6/20/2012	12:50	7.50	0.648	28.58	7.25	177	420
LR 3	6/20/2012	13:39	7.72	0.831	27.95	7.07	58	530
LR 4	6/20/2012	14:17	7.76	0.622	27.76	8.75	165	400
LR 5	6/20/2012	14:45	7.65	0.715	26.94	7.63	162	460
LR 6	6/20/2012	15:02	7.39	0.772	24.85	8.07	178	500
LR 7	6/20/2012	15:49	7.42	0.675	28.31	6.38	182	430
LR 8	6/20/2012	16:22	7.42	0.691	29.24	3.75	89	440

LR 9	6/20/2012	16:28	7.53	0.715	27.29	3.85	80	450
LR 10	6/20/2012	17:28	7.38	0.621	25.10	7.22	182	400

Table 5. Discharge measurements recorded at each sampling site in the Bayou de Chien (BdC), Clarks River (CR), South Elkhorn Creek (SEC), Banklick Creek (BC), and Licking River (LR) watersheds. NMF indicates no measureable flow. NR indicates that a discharge measurement was not recorded.

Site Name	Date	Time	Marsh-McBirney Discharge (cfs)	USGS Gage Discharge (cfs)	USGS Gage Station
BdC 1	6/25/2012	14:25	7.4		
BdC 2	6/25/2012	15:22		18.00	07024000
BdC 3	6/25/2012	15:56	NR		
BdC 4	6/25/2012	16:30	NR		
CR 1	6/25/2012	8:12	NMF		
CR 2	6/25/2012	9:09	0.1		
CR 3	6/25/2012	9:39	1.2		
CR 4	6/25/2012	10:38		2.80	03610200
CR 5	6/25/2012	11:31	7.5		
CR 6	6/25/2012	12:24	NR		
SEC 1	6/19/2012	8:19	2.6		
SEC 2	6/19/2012	9:18	22.6		
SEC 3	6/19/2012	10:10		1.60	03289193
SEC 4	6/19/2012	10:33		34.00	03289200
SEC 5	6/19/2012	11:09	2.0		
SEC 6	6/19/2012	11:42	0.3		
SEC 7	6/19/2012	13:09	4.3		
SEC 8	6/19/2012	14:02		37.00	03289300
SEC 9	6/19/2012	14:30	0.3		
SEC 10	6/19/2012	15:00	NR		
SEC 11	6/19/2012	15:38	57.9		
BC 1	6/27/2012	10:35	NMF		
BC 2	6/27/2012	10:54	NMF		
BC 3	6/27/2012	11:26	0.1		
BC 4	6/27/2012	11:59		0.02	03254550
BC 5	6/27/2012	13:32	0.2		
BC 6	6/27/2012	14:07	0.4		
LR 1	6/20/2012	12:28	3.3		
LR 2	6/20/2012	12:50	0.8		
LR 3	6/20/2012	13:39	0.1		
LR 4	6/20/2012	14:17	NMF		

LR 5	6/20/2012	14:45	NMF		
LR 6	6/20/2012	15:02	5.1		
LR 7	6/20/2012	15:49		12.00	03248300
LR 8	6/20/2012	16:22	NR		
LR 9	6/20/2012	16:28	NR		
LR 10	6/20/2012	17:28	11.2		

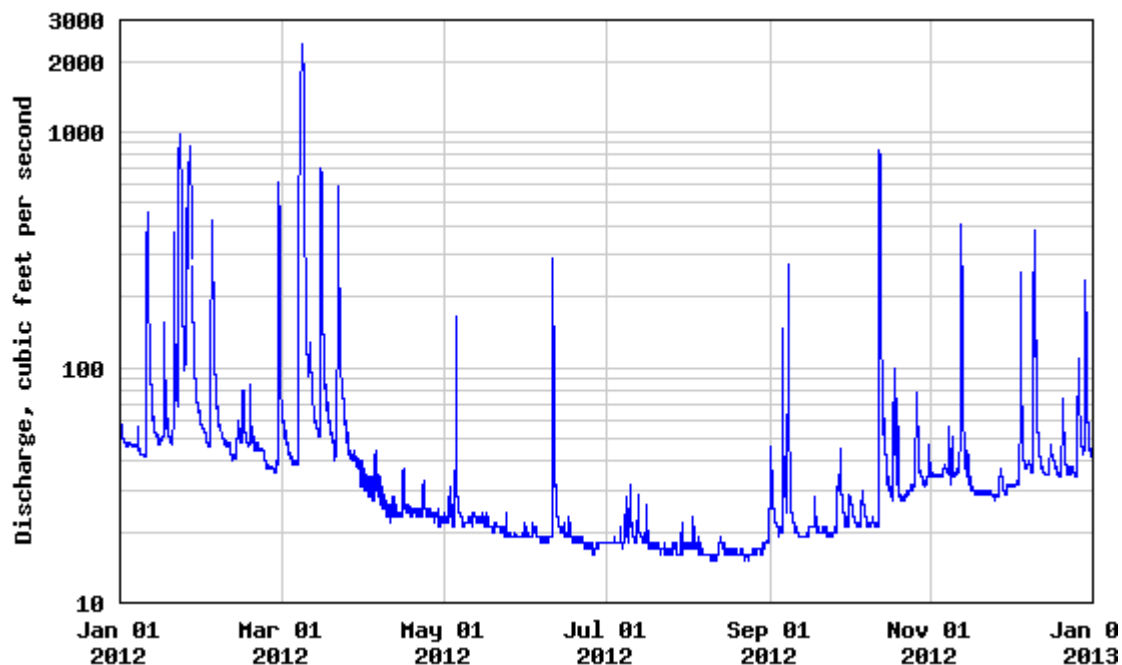


Figure 4. Hydrograph for Bayou de Chien site 2 (Fig. 3) showing low flow conditions during sampling. Stream sample was collected on June 25, 2012. Hydrograph dates range from January 1, 2012 to December 31, 2012. Data for the hydrograph are from the USGS stream gage station located at site 2 (Table 3). The hydrograph was obtained from the USGS National Water Information System Web site [http://ky.water.usgs.gov/hyd_data/gagemap_2012_links.pdf].

Site 4 is the only site that had an estradiol concentration above the MDL of 3.0 ppt (Table 6). Both analytical reps for site 4 were above the estradiol MDL. Only one analytical rep for the duplicate sample collected at site 4 was above the estradiol MDL (Table 6). All samples were below the fluoroquinolones MDL of 0.025 ppb (Table 6).

Table 6. Estradiol and fluoroquinolones analytical results for field samples and duplicate sample collected in the Bayou de Chien watershed.

Sample Name	Date	Time	Estradiol (ppt)	Fluoroquinolones (ppb)
Bayou de Chien 1 a	6/25/2012	14:25	< 3.0	< 0.025
Bayou de Chien 1 b	6/25/2012	14:25	< 3.0	< 0.025
Bayou de Chien 2 a	6/25/2012	15:22	< 3.0	< 0.025
Bayou de Chien 2 b	6/25/2012	15:22	< 3.0	< 0.025
Bayou de Chien 3 a	6/25/2012	15:56	< 3.0	< 0.025
Bayou de Chien 3 b	6/25/2012	15:56	< 3.0	< 0.025
Bayou de Chien 4 a	6/25/2012	16:30	3.3	< 0.025
Bayou de Chien 4 b	6/25/2012	16:30	4.0	< 0.025
Bayou de Chien Duplicate 4 c	6/25/2012	16:31	3.3	< 0.025
Bayou de Chien Duplicate 4 d	6/25/2012	16:31	< 3.0	< 0.025

Clarks River Watershed

The head waters of the Clarks River begin in northern Henry County, Tennessee and flows north through Calloway, Marshall, and McCracken Counties in Kentucky before reaching the Tennessee River near Paducah, Kentucky (Fig. 1). The dominate land-cover types in the watershed are cultivated (42 percent) and forest (32 percent) (Table 1; Fig. 5). There are five mapped municipal WWTP outfalls in the watershed (Table 1). Sample sites 2 and 3 are located on Bee Creek upstream and downstream, respectively, of the Murray WWTP outfall (Table 3; Fig. 5). The population of Murray is approximately 18,000 people. Sample sites 5 and 6 are located on Clarks River upstream and downstream, respectively, of the Benton WWTP outfall (Table 3; Fig. 5). The population of Benton is approximately 4,400 people.

Six sites were sampled in the watershed (Fig. 5). Field measurement data for all six sites are shown in Table 4. Discharge measurements were obtained for four of the six sites (Table 5). The stream at site 1 exhibited no measureable flow. In-stream discharge measurements were taken at sites 2, 3, and 5 (Table 5). A discharge measurement for site 4 was obtained from a USGS stream gage station installed at site 4 (Table 5). A discharge measurement was not taken at site 6 because of deep water (> 4 ft). On the day of sampling, June 25, 2012, the discharge at site 4 was 2.80 cfs (Table 5). As depicted in Figure 6, Clarks River samples were collected during low flow conditions.

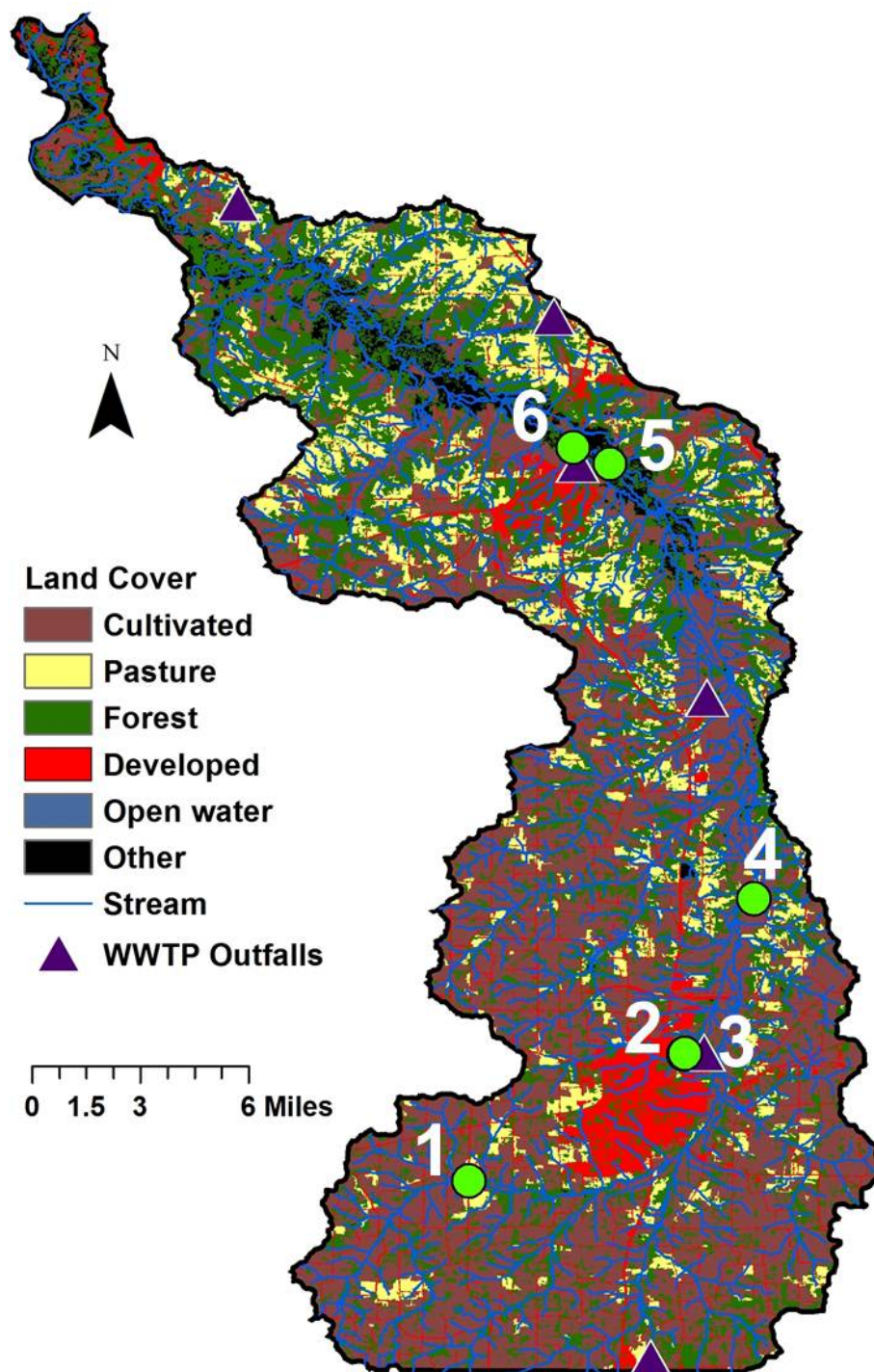


Figure 5. Map of the Clarks River watershed with land cover (Homer and others, 2007). Green circles are sampling site locations with corresponding sample number.

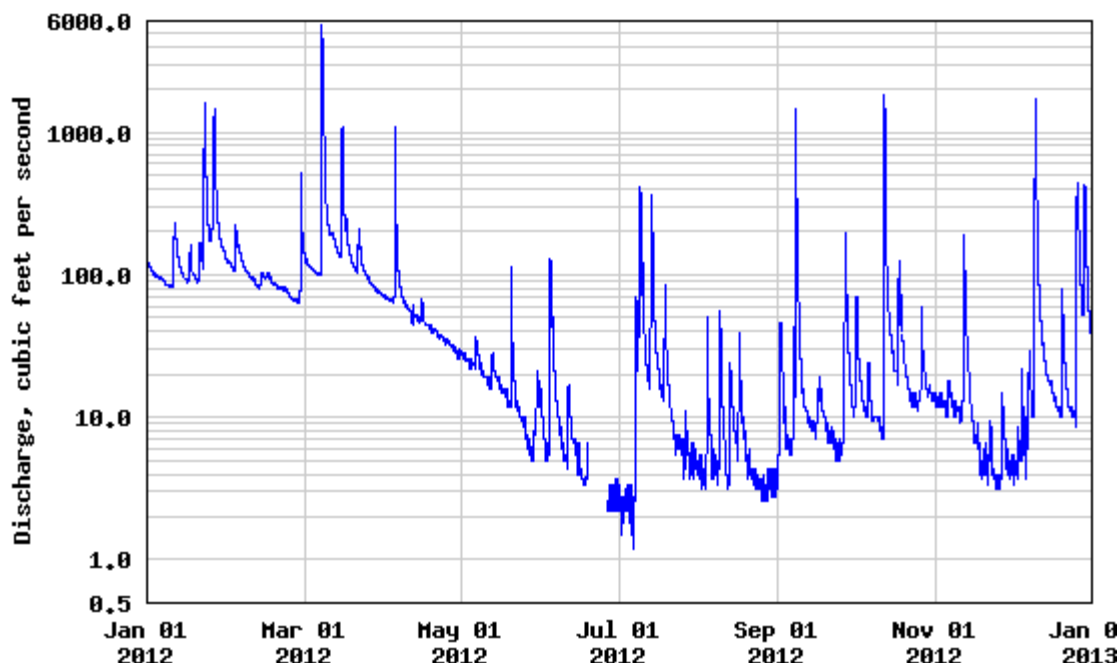


Figure 6. Hydrograph for Clarks River site 4 (Fig. 5) showing low flow conditions during sampling. Stream sample was collected on June 25, 2012. Hydrograph dates range from January 1, 2012 to December 31, 2012. Data for the hydrograph are from the USGS stream gage station located at site 4 (Table 3). The hydrograph was obtained from the USGS National Water Information System Web site [http://ky.water.usgs.gov/hyd_data/gagemap_2012_links.pdf].

Site 4 was the only site that had an estradiol concentration above the MDL of 3.0 ppt (Table 7). Only one analytical rep for site 4 was above the estradiol MDL (Table 7). Estradiol duplicate reps for site 4 were below the MDL (Table 7). The possible source of estradiol seen at site 4 is the Murray WWTP outfall, which is located approximately four miles upstream of site 4. The estradiol concentration for site 3, located approximately 350 ft downstream of the Murray outfall, was below the MDL.

Site 3 was the only site that had a fluoroquinolones concentration above the MDL of 0.025 ppb (Table 7). Only one analytical rep for site 3 was above the fluoroquinolones MDL (Table 7). The source of fluoroquinolones seen at site 3 is most likely the Murray WWTP outfall located upstream of site 3. Blank samples were below the MDL for fluoroquinolones and estradiol (Table 7).

Table 7. Estradiol and fluoroquinolones analytical results for field samples, duplicate sample, and blank sample collected in the Clarks River watershed.

Sample Name	Date	Time	Estradiol (ppt)	Fluoroquinolones (ppb)
Clarks River 1 a	6/25/2012	8:12	< 3.0	< 0.025
Clarks River 1 b	6/25/2012	8:12	< 3.0	< 0.025
Clarks River 2 a	6/25/2012	9:09	< 3.0	< 0.025
Clarks River 2 b	6/25/2012	9:09	< 3.0	< 0.025
Clarks River 3 a	6/25/2012	9:39	< 3.0	< 0.025
Clarks River 3 b	6/25/2012	9:39	< 3.0	0.032
Clarks River 4 a	6/25/2012	10:38	3.9	< 0.025
Clarks River 4 b	6/25/2012	10:38	< 3.0	< 0.025
Clarks River Duplicate 4 c	6/25/2012	10:39	< 3.0	< 0.025
Clarks River Duplicate 4 d	6/25/2012	10:39	< 3.0	< 0.025
Clarks River 5 a	6/25/2012	11:31	< 3.0	< 0.025
Clarks River 5 b	6/25/2012	11:31	< 3.0	< 0.025
Clarks River 6 a	6/25/2012	12:24	< 3.0	< 0.025
Clarks River 6 b	6/25/2012	12:24	< 3.0	< 0.025
Clarks River Blank 1 a	6/25/2012	7:56	< 3.0	< 0.025
Clarks River Blank 1 b	6/25/2012	7:56	< 3.0	< 0.025

South Elkhorn Creek Watershed

The South Elkhorn Creek watershed is located in northern Jessamine, northwestern Fayette, northeastern Woodford, southwestern Scott and southeastern Franklin Counties (Fig. 1). South Elkhorn Creek rises in western Fayette County and flows northwesterly until it reaches Elkhorn Creek in Franklin County. The dominate land-cover types in the watershed is pasture (63 percent) and developed (24 percent) (Table 1). Municipal WWTP outfalls associated with the cities of Midway and Lexington are the only two outfalls mapped in the watershed (Table 1; Fig. 7). The population of Midway and Lexington is approximately 1,600 and 300,000 people, respectively. The Lexington outfall is located between sites 1 and 2 on Town Branch in Fayette County (Fig. 7). The Midway outfall is located between sites 9 and 10 on Lee Branch in Woodford County (Fig. 7).

Eleven sites were sampled in the watershed (Fig. 7). In-stream samples and field measurement data were collected at all 11 sites. Field measurements are listed in Table 4.

In-stream discharge measurements were taken for sites 1, 2, 5, 6, 7, 9, and 11 (Table 5). Because of heavy vegetation growth in the stream a discharge measurement was not taken at site 10. A discharge measurement for sites 3, 4, and 8 were obtained from USGS stream gage stations installed at each site (Table 5). On the day of sampling, June 19, 2012, the discharge at site 8 was 37.00 cfs (Table 5). As depicted in Figure 8, South Elkhorn Creek watershed samples were collected during low flow conditions.

Seven of the 11 sites had estradiol concentrations greater than the MDL (Table 8). The highest estradiol concentration detected in all five watersheds was at site 2 where the concentration was five times the MDL (Table 8). Site 2 is located on Town Branch approximately 250 ft from the Lexington outfall. The estradiol concentration was also greater than the MDL at site 4 (Table 8), which is also located on Town Branch approximately four miles downstream of the Lexington outfall (Fig. 7). Effluent from the Lexington outfall is believed to be the source of the estradiol detected at sites 2 and 4. Samples collected from sites 5 and 7 had estradiol concentrations greater than the MDL (Table 8). Sites 5 and 7 are located on South Elkhorn Creek where the predominant land cover is pasture (Fig. 7). There are no mapped WWTP outfalls upstream of either site 5 or 7. A possible source of estradiol detected at sites 5 and 7 is surface runoff from surrounding pasture fields where animals graze. Precipitation data taken from the Kentucky Mesonet Web site shows that between June 11 and 17 there was 0.74 in. of rain in Fayette County. Samples were collected on June 19, which means that runoff from the fields could have entered the stream before sampling occurred. In addition, discharge from limestone springs, which are prevalent in this area, could also be sources of estradiol-rich water entering the stream. In conjunction with surface runoff and spring discharge, low flow conditions could have allowed the estradiol to be concentrated enough in the stream water to be detected. The sample collected from site 8, located on South Elkhorn Creek downstream of sites 2, 4, 5, and 7 (Fig. 7), had an estradiol concentration greater than the MDL (Table 8). The source of estradiol detected at site 8 is most likely a combination of effluent from the Lexington outfall, estradiol-rich surface runoff water from surrounding pasture fields, and potentially estradiol-rich spring water discharging from limestone bedrock. At site 10, located downstream of the Midway WWTP outfall (Fig. 7), out of four reps only one rep was greater than the MDL (Table 8). Estradiol was detected at site 11 (Table 8), which is located approximately 600 ft from where South Elkhorn Creek reaches Elkhorn Creek (Fig. 7). Like site 8, the source of estradiol detected at site 11 is most likely a combination of effluent from WWTP outfalls, surface runoff from surrounding pasture fields, and limestone spring discharge.

Fluoroquinolones concentrations at site 2 were nine times greater than the MDL (Table 8). This is the highest concentration detected in any of the five watersheds sampled. Like estradiol, effluent from the Lexington outfall is believed to be the source of the elevated fluoroquinolones. Fluoroquinolones concentrations at all other sites were less than the MDL (Table 8).

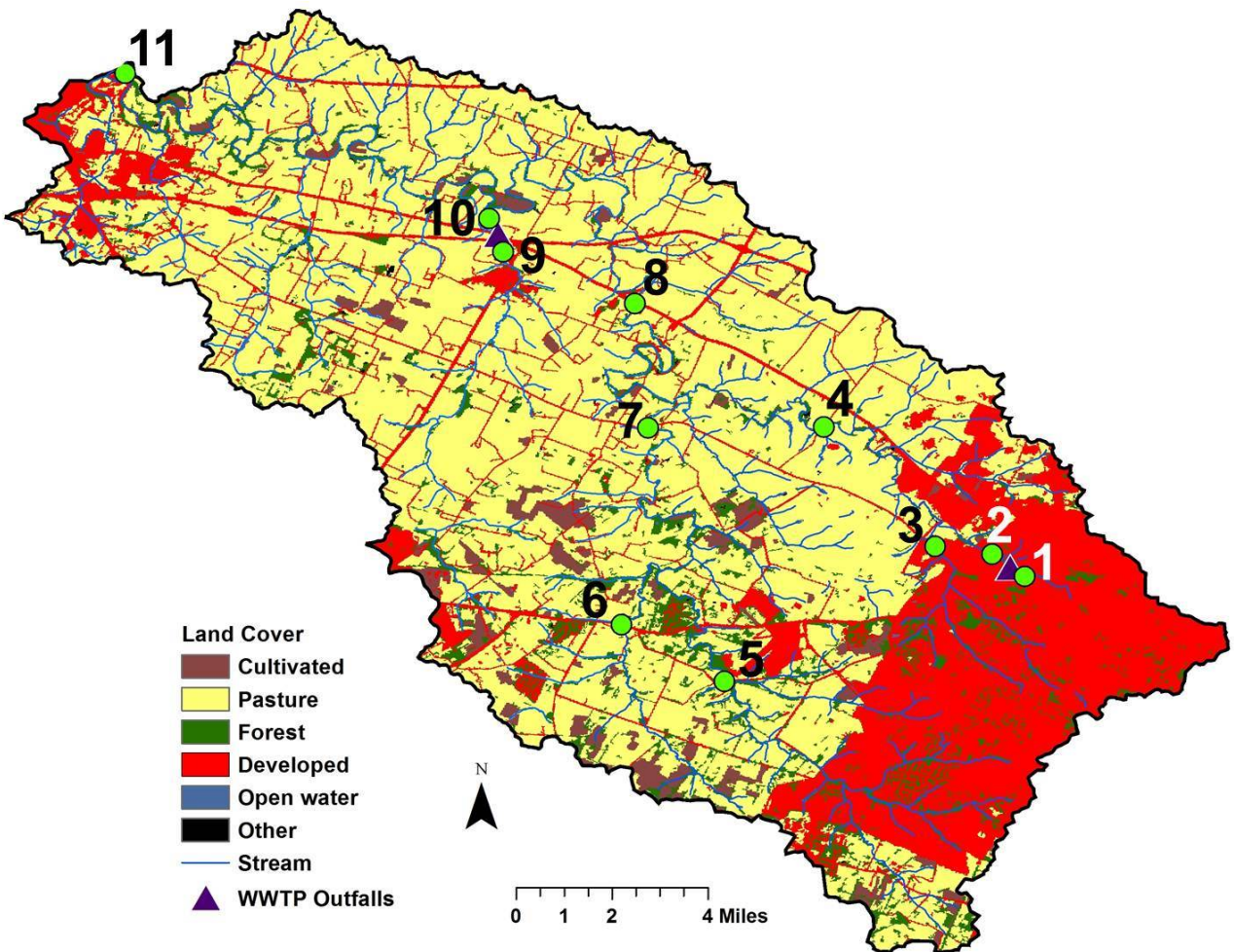


Figure 7. Map of the South Elkhorn Creek watershed with land cover (Homer and others, 2007). Green circles are sampling site locations with corresponding sample number.

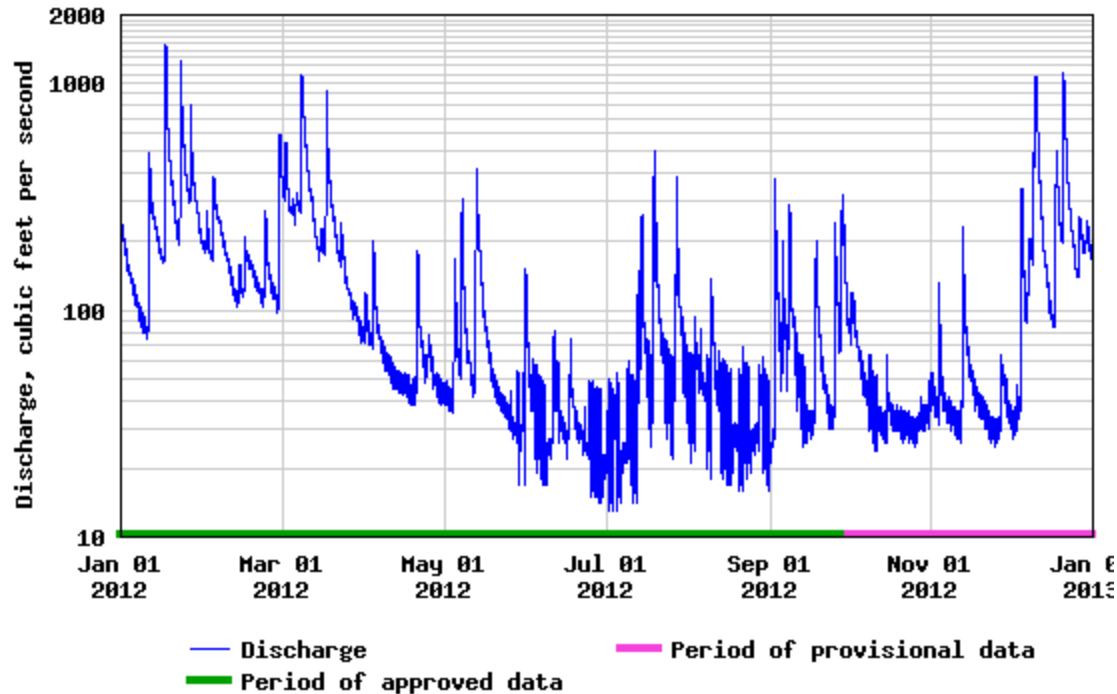


Figure 8. Hydrograph for South Elkhorn Creek site 8 (Fig. 7) showing low flow conditions during sampling. Stream sample was collected on June 19, 2012. Hydrograph dates range from January 1, 2012 to December 31, 2012. Data for the hydrograph are from the USGS stream gage station located at site 4 (Table 3). The hydrograph was obtained from the USGS National Water Information System Web site [http://ky.water.usgs.gov/hyd_data/gagemap_2012_links.pdf].

Banklick Creek Watershed

Banklick Creek watershed is located in eastern Boone County and central Kenton County (Fig. 1). Banklick Creek rises in eastern Boone County and flows through Kenton County before reaching the Licking River south of Latonia, Kentucky. The dominate land-cover types in the watershed are developed (36 percent), forest (36 percent), and pasture (24 percent) (Table 1; Fig. 9). There are six mapped WWTP outfalls in the watershed, but none are associated with a municipal WWTP (Fig. 9).

Six sites were sampled in the Banklick Creek watershed (Fig. 9). Because of steep stream banks at site 2, collecting an in-stream sample was not possible. Therefore, site 2 was sampled from a highway bridge (Table 3). Field measurement data collected at five of the sites are shown in Table 4. Field measurements were not taken at site 2 because of the inability to take in-stream measurements.

Because of very low flow conditions at site 1 and the inability to access the stream at site 2 it was not possible to take in-stream discharge measurements at these sites (Table 5). In-stream discharge measurements were taken at sites 3, 5, and 6 (Table 5). A discharge measurement for site 4 was obtained from a USGS stream gage station in operation at site 4 (Table 5). On the day of sampling, June 27, 2012, the discharge at site 4 was 0.02 cfs (Table 5). As depicted in Figure 10, Banklick Creek watershed samples were collected during low flow conditions.

Table 8. Estradiol and fluoroquinolones analytical results for field samples, duplicate samples, and blank sample collected in the South Elkhorn Creek watershed.

Sample Name	Date	Time	Estradiol (ppt)	Fluoroquinolones (ppb)
South Elkhorn Creek 1 a	6/19/2012	8:19	< 3.0	< 0.025
South Elkhorn Creek 1 b	6/19/2012	8:19	< 3.0	< 0.025
South Elkhorn Creek 2 a	6/19/2012	9:18	15.0	0.202
South Elkhorn Creek 2 b	6/19/2012	9:18	15.5	0.256
South Elkhorn Creek 3 a	6/19/2012	10:10	< 3.0	< 0.025
South Elkhorn Creek 3 b	6/19/2012	10:10	< 3.0	< 0.025
South Elkhorn Creek 4 a	6/19/2012	10:33	4.3	< 0.025
South Elkhorn Creek 4 b	6/19/2012	10:33	4.8	< 0.025
South Elkhorn Creek Duplicate 4 c	6/19/2012	10:34	4.7	< 0.025
South Elkhorn Creek Duplicate 4 d	6/19/2012	10:34	6.2	< 0.025
South Elkhorn Creek 5 a	6/19/2012	11:09	< 3.0	< 0.025
South Elkhorn Creek 5 b	6/19/2012	11:09	5.0	< 0.025
South Elkhorn Creek 6 a	6/19/2012	11:42	< 3.0	< 0.025
South Elkhorn Creek 6 b	6/19/2012	11:42	< 3.0	< 0.025
South Elkhorn Creek 7 a	6/19/2012	13:09	4.8	< 0.025
South Elkhorn Creek 7 b	6/19/2012	13:09	5.5	< 0.025
South Elkhorn Creek 8	6/19/2012	14:02	3.8	< 0.025
South Elkhorn Creek 8 b	6/19/2012	14:02	4.2	< 0.025
South Elkhorn Creek 9 a	6/19/2012	14:30	< 3.0	< 0.025
South Elkhorn Creek 9 b	6/19/2012	14:30	< 3.0	< 0.025
South Elkhorn Creek 10 a	6/19/2012	15:00	< 3.0	< 0.025
South Elkhorn Creek 10 b	6/19/2012	15:00	< 3.0	< 0.025
South Elkhorn Creek Duplicate 10 c	6/19/2012	15:01	3.6	< 0.025
South Elkhorn Creek Duplicate 10 d	6/19/2012	15:01	< 3.0	< 0.025
South Elkhorn Creek 11 a	6/19/2012	15:38	4.1	< 0.025
South Elkhorn Creek 11 b	6/19/2012	15:38	< 3.0	< 0.025
South Elkhorn Creek Blank 1 a	6/19/2012	8:02	< 3.0	< 0.025
South Elkhorn Creek Blank 1 b	6/19/2012	8:02	< 3.0	< 0.025

Sites 1 and 4 had estradiol concentrations above the MDL (Table 9). Since there are no municipal WWTP outfalls in this watershed, the source of the estradiol in these samples is unknown. Both analytical reps for site 1 were more than three times the MDL, but only one of the reps for site 4 was above the MDL (Table 9). Both duplicate sample reps for site 4 were also below the MDL (Table 9). All samples were below the fluoroquinolones MDL (Table 9). Estradiol and fluoroquinolones concentrations in the blank sample were below the MDL (Table 9).

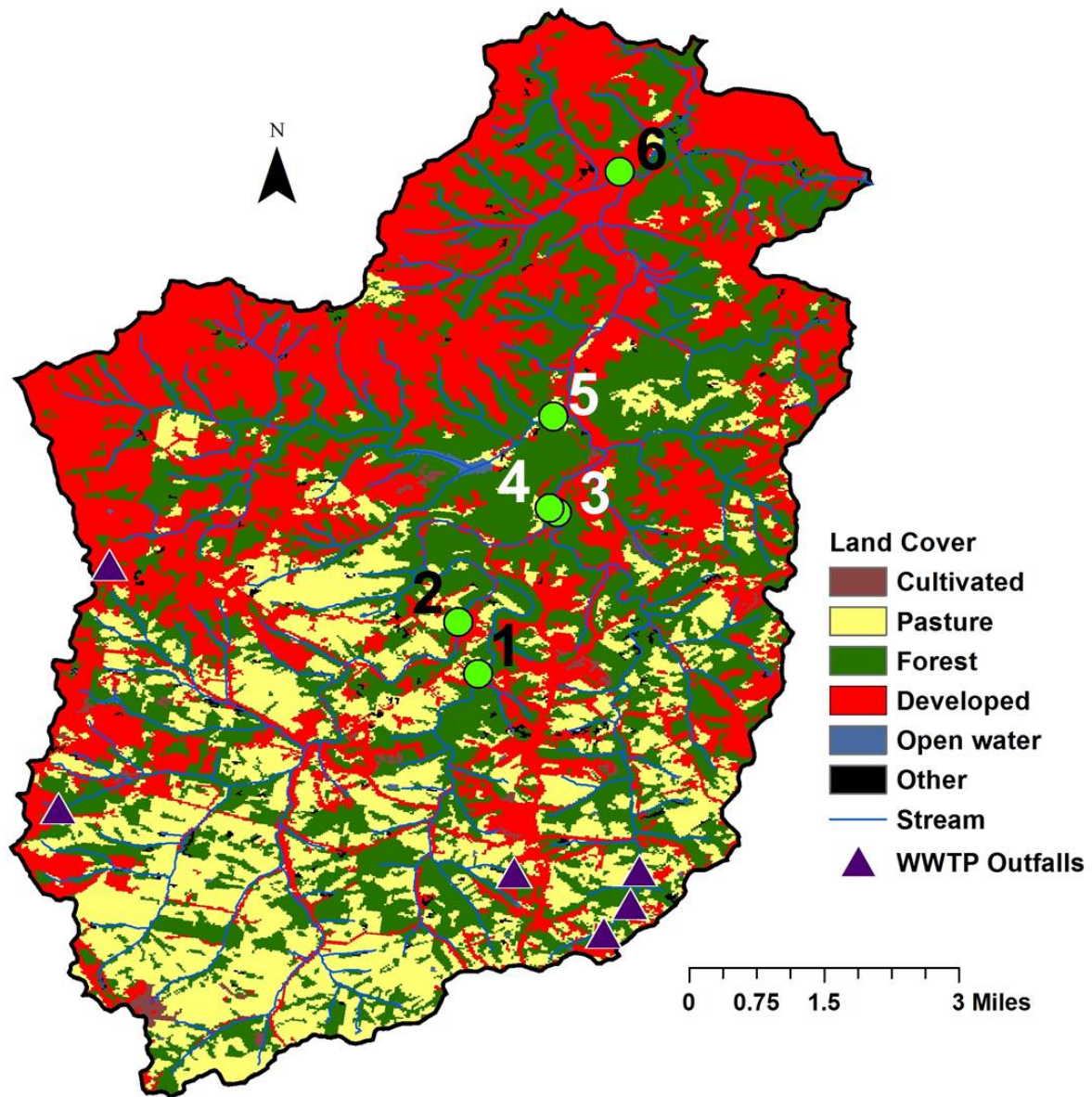


Figure 9. Map of the Banklick Creek watershed with land cover (Homer and others, 2007). Green circles are sampling site locations with corresponding sample number.

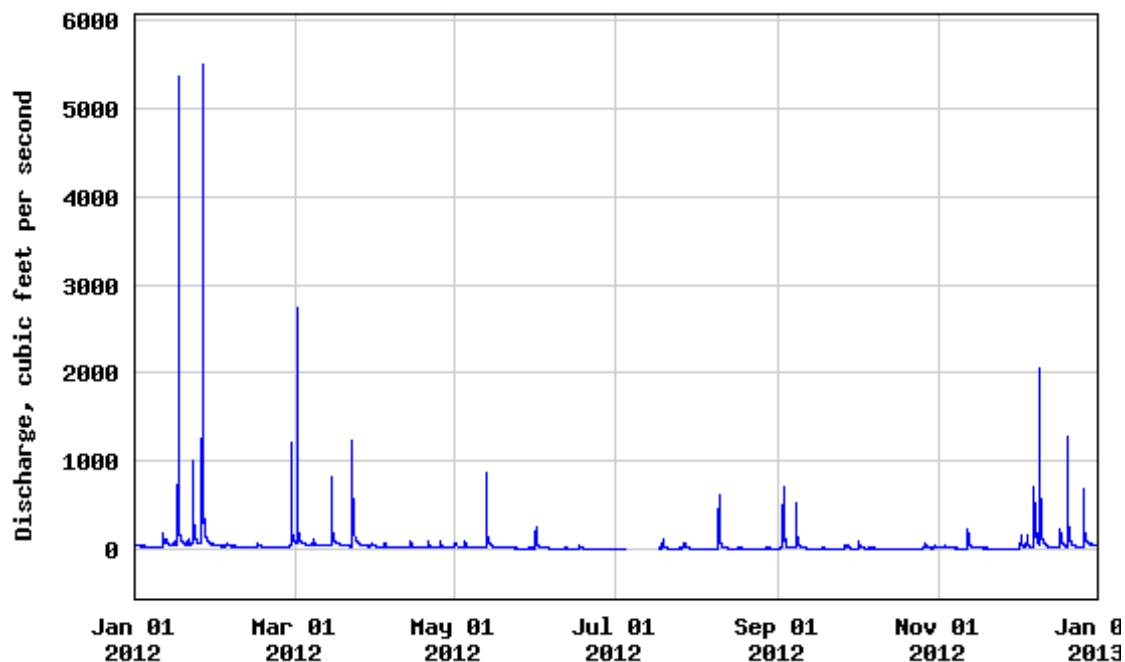


Figure 10. Hydrograph for Banklick River site 4 (Fig. 9) showing low flow conditions during sampling. Stream sample was collected on June 27, 2012. Hydrograph dates range from January 1, 2012 to December 31, 2012. Data for the hydrograph are from the USGS stream gage station located at site 4 (Table 3). The hydrograph was obtained from the USGS National Water Information System Web site [http://ky.water.usgs.gov/hyd_data/gagemap_2012_links.pdf].

Table 9. Estradiol and fluoroquinolones analytical results for field samples, duplicate sample, and blank sample collected in the Banklick Creek watershed.

Sample Name	Date	Time	Estradiol (ppt)	Fluoroquinolones (ppb)
Banklick Creek 1 a	6/27/2012	10:35	11.0	< 0.025
Banklick Creek 1 b	6/27/2012	10:35	11.1	< 0.025
Banklick Creek 2 a	6/27/2012	10:54	< 3.0	< 0.025
Banklick Creek 2 b	6/27/2012	10:54	< 3.0	< 0.025
Banklick Creek 3 a	6/27/2012	11:26	< 3.0	< 0.025
Banklick Creek 3 b	6/27/2012	11:26	< 3.0	< 0.025
Banklick Creek 4 a	6/27/2012	11:59	< 3.0	< 0.025
Banklick Creek 4 b	6/27/2012	11:59	3.9	< 0.025
Banklick Creek Duplicate 4 c	6/27/2012	12:00	< 3.0	< 0.025
Banklick Creek Duplicate 4 d	6/27/2012	12:00	< 3.0	< 0.025
Banklick Creek 5 a	6/27/2012	13:32	< 3.0	< 0.025
Banklick Creek 5 b	6/27/2012	13:32	< 3.0	< 0.025
Banklick Creek 6 a	6/27/2012	14:07	< 3.0	< 0.025
Banklick Creek 6 b	6/27/2012	14:07	< 3.0	< 0.025

Banklick Creek Blank 1 a	6/27/2012	10:23	< 3.0	< 0.025
Banklick Creek Blank 1 b	6/27/2012	10:23	< 3.0	< 0.025

Licking River Watershed

The Licking River watershed designated in this study is the HUC 11-digit watershed associated with the headwaters of the Licking River flowing through Magoffin County, Kentucky (Figures 1 and 11). The dominate land cover in the watershed is forest (82 percent) (Table 1). The City of Salyersville's WWTP outfall is the only mapped outfall in the watershed. The outfall is located between sample sites 8 and 9 (Fig. 11). The population of Salyersville is approximately 1,900 people.

Ten sites were sampled in the watershed (Fig. 11). In-stream samples and field measurement data were collected at all 10 sites. Field measurements are listed in Table 4.

In-stream discharge measurements were taken for sites 1, 2, 3, 6, and 10 (Table 5). Because of very low flow conditions and a heavily rocked stream bed discharge measurements were not taken at sites 4 and 5. Discharge measurements were also not taken at sites 8 and 9 because of the inability to cross the stream because of the depth of water (> 3 ft) and a very muddy streambed. A discharge measurement for site 7 was obtained from a USGS stream gage station installed at site 7 (Table 5). On the day of sampling, June 20, 2012, the discharge at site 7 was 12.00 cfs (Table 5). As depicted in Figure 12, Licking River watershed samples were collected during low flow conditions.

Sites 7 and 9 were the only sites with estradiol concentrations above the MDL (Table 10). Only one site 7 analytical rep was above the MDL, while both site 9 reps were greater than the MDL (Table 10). Only one duplicate sample rep for site 9 was above the MDL (Table 10). The source of estradiol present in the sample collected from site 7 is unknown, possibly surface runoff from pasture fields where animals graze (Fig. 11). The source of estradiol seen in the sample collected from site 9 is most likely effluent from the Salyersville WWTP outfall. Site 9 is located approximately 100 ft downstream of the outfall.

With the exception of the site 9 sample, fluoroquinolones concentrations in all other samples were below the MDL (Table 10). Like estradiol, the most likely source of fluoroquinolones seen in the site 9 sample is effluent from the Salyersville WWTP outfall.

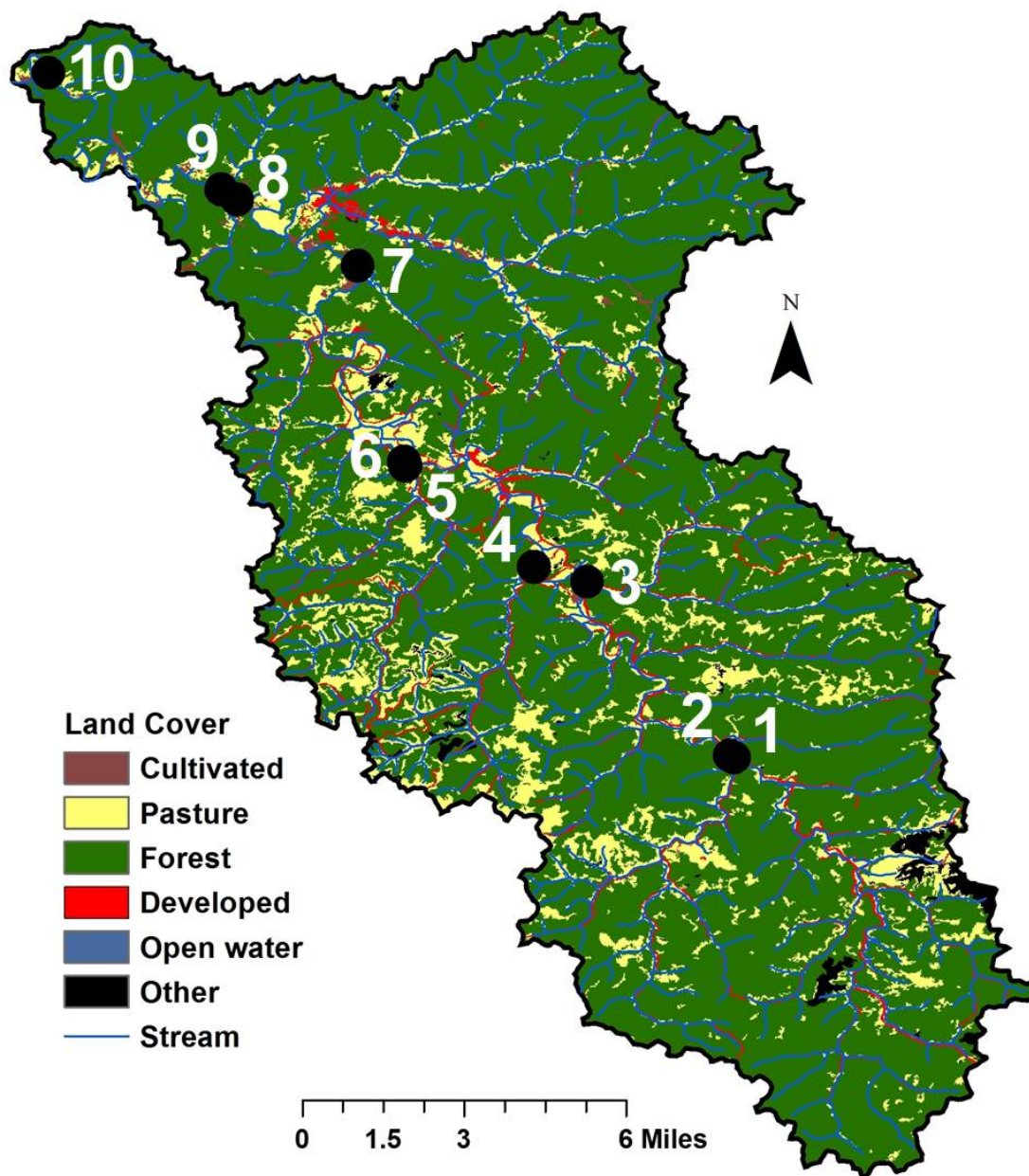


Figure 11. Map of the Licking River watershed with land cover (Homer and others, 2007). Black circles are sampling site locations with corresponding sample number

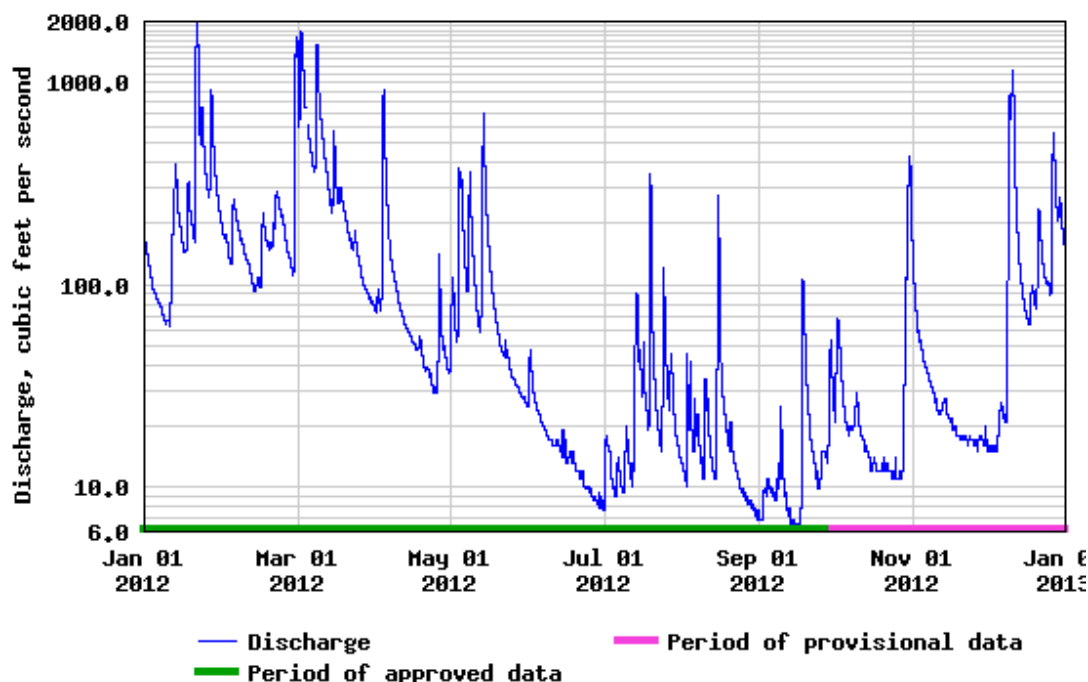


Figure 12. Hydrograph for Licking River site 7 (Fig. 11) showing low flow conditions during sampling. Stream sample was collected on June 20, 2012. Hydrograph dates range from January 1, 2012 to December 31, 2012. Data for the hydrograph are from the USGS stream gage station located at site 4 (Table 3). The hydrograph was obtained from the USGS National Water Information System Web site [http://ky.water.usgs.gov/hyd_data/gagemap_2012_links.pdf].

Summary

Of the 37 samples collected in five watersheds 13 (35 percent) contained estradiol greater than the MDL and three (8 percent) contained fluoroquinolones greater than the MDL. The highest estradiol and fluoroquinolones concentrations, 15.5 ppt and 0.256 ppb, respectively, were in samples collected within 250 ft of a municipal WWTP outfall. Estradiol and fluoroquinolones concentrations are generally higher in watersheds where the percentage of developed land cover is higher.

Results from this study indicate that potential sources of estradiol to surface water in Kentucky are municipal WWTP outfalls and surface runoff from pasture fields where animals graze. Additional research is needed to better determine these sources, particularly surface runoff from pasture fields and manure amended fields, and the input of estradiol from springs discharging from limestone bedrock. Additional sampling and compound specific analysis are needed to better determine the actual concentrations of estrogen and fluoroquinolones compounds in surface water in Kentucky.

Table 10. Estradiol and fluoroquinolones analytical results for field samples, duplicate samples, and blank sample collected in the Licking River watershed.

Sample Name	Date	Time	Estradiol (ppt)	Fluoroquinolones (ppb)
Licking River 1 a	6/20/2012	12:28	< 3.0	< 0.025
Licking River 1 b	6/20/2012	12:28	< 3.0	< 0.025
Licking River 2 a	6/20/2012	12:50	< 3.0	< 0.025
Licking River 2 b	6/20/2012	12:50	< 3.0	< 0.025
Licking River 3 a	6/20/2012	13:39	< 3.0	< 0.025
Licking River 3 b	6/20/2012	13:39	< 3.0	< 0.025
Licking River 4 a	6/20/2012	14:17	< 3.0	< 0.025
Licking River 4 b	6/20/2012	14:17	< 3.0	< 0.025
Licking River 5 a	6/20/2012	14:45	< 3.0	< 0.025
Licking River 5 b	6/20/2012	14:45	< 3.0	< 0.025
Licking River Duplicate 5 c	6/20/2012	14:46	< 3.0	< 0.025
Licking River Duplicate 5 d	6/20/2012	14:46	< 3.0	< 0.025
Licking River 6 a	6/20/2012	15:02	< 3.0	< 0.025
Licking River 6 b	6/20/2012	15:02	< 3.0	< 0.025
Licking River 7 a	6/20/2012	15:49	4.8	< 0.025
Licking River 7 b	6/20/2012	15:49	< 3.0	< 0.025
Licking River 8 a	6/20/2012	16:22	< 3.0	< 0.025
Licking River 8 b	6/20/2012	16:22	< 3.0	< 0.025
Licking River 9 a	6/20/2012	16:28	3.1	0.071
Licking River 9 b	6/20/2012	16:28	3.5	0.032
Licking River Duplicate 9 c	6/20/2012	16:29	< 3.0	< 0.025
Licking River Duplicate 9 d	6/20/2012	16:29	5.6	< 0.025
Licking River 10 a	6/20/2012	17:28	< 3.0	< 0.025
Licking River 10 b	6/20/2012	17:28	< 3.0	< 0.025
Licking River Blank 1 a	6/20/2012	17:51	< 3.0	< 0.025
Licking River Blank 1 b	6/20/2012	17:51	< 3.0	< 0.025

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Determining groundwater flow velocities and discharge rate at the Kentucky Horse Park Royal Spring conduit monitoring station

Basic Information

Title:	Determining groundwater flow velocities and discharge rate at the Kentucky Horse Park Royal Spring conduit monitoring station
Project Number:	2012KY208B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	KY 6th
Research Category:	Ground-water Flow and Transport
Focus Category:	Hydrology, Groundwater, None
Descriptors:	karst, cave, tracer injection
Principal Investigators:	James C. Currens

Publications

1. Agouridis, Carmen, James Currens, and James Fox, 2013, Karst Hydrogeology Investigations in the Royal Spring Groundwater Basin, Internal Status Report, College of Agriculture and Kentucky Geological Survey, University of Kentucky, 35 p
2. Currens, James and M. Farwell, 2013, Groundwater Discharge at the Kentucky Horse Park KWIS Station, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 17-18

Determining Groundwater Flow Velocities and Discharge Rate at the Kentucky Horse Park Royal Spring Conduit Monitoring Station

Problem and Research Objectives

The purpose of establishing the Karst Water Instrumentation System (KWIS) groundwater station at the Kentucky Horse Park is to measure the mass flux of several contaminants as they exit Fayette County. The KWIS is in the Royal Spring springshed, which has a total drainage area of approximately 6,070 hectares of which 3,700 ha are upgradient of the site. The headwaters of the springshed are in the east-central part of Lexington. Much of the land use in the basin is urban, although there are some areas of agriculture, suburbs and light industry. The KWIS station is at a critical site in the springshed, because the surface and underground flow splits at this location in the Horse Park (Fig. 1). The site is also near the Scott-Fayette County boundary. The cave into which the KWIS instrumentation is installed was discovered after extensive electrical-resistivity surveying and drilling 20 wells in the vicinity of the KWIS site.

The objective of the research was to utilize quantitative dye tracing to independently determine the cross section and the discharge at the KWIS. To accomplish this, a method was needed to continuously monitor discharge in the cave. The cross-sectional area of the cave, the velocity profile, and therefore the discharge, are challenging to determine because the cave cannot be entered (as it is perennially flooded at this location). Typically, there is 7 m (20 ft) of water above the cave ceiling in the wells, and during high-flow events, when there is water flowing on the surface in Cane Run, there may be 15 m (50 ft) or more of water above the cave. Because sudden increases in head in the central conduit does not instantaneously translate to a rise in water levels in wells flanking the conduit, the cave behaves as a semiconfined flow system. There are a minimum of six large swallow holes and many smaller ones along Cane Run



Figure 1. Vicinity map of the Kentucky Horse Park showing the location of the KyHP monitoring station.

from the Kentucky Geological Survey (KGS) Well Sample and Core Library downstream to the Eclipse Road bridge immediately west of the indoor arena at the Kentucky Horse Park. KGS has previously inspected the cave with a submersible borehole video camera that was modified to tilt horizontally. Doppler sonar has also been used with limited success. There are significant uncertainties associated with both methods because of data definition and the possibility of discharge bypassing the recovery site in flanking anastomotic conduits.

The drought that occurred in the lower 48 states beginning in the spring of 2012 made collecting meaningful data impossible. After some late summer rain, traces conducted in the early fall of 2012 were for the lowest discharge determined. The flow conditions improved by spring 2013, and we completed the field work. Eleven attempts were made to conduct quantitative traces. Two were omitted from further analysis because only one tracer was used. Another trace was not used because a leak developed in the tub used to dilute the tracer, and the fourth was excluded from the data set because of flow being cut off when the suction hose vacuumed itself against the wall of the tub. Therefore, seven quantitative groundwater tracer experiments resulted in satisfactory data (Table 1).

Methodology

Constant flux tracer injection was used to determine the discharge at the KWIS station (Fig. 2). A spike injection of sodium fluorescein was used to determine the velocity along the course of the flow route. Rhodamine WT was used for the constant flux tracer because the mass of dye in the concentrate liquid is accurately known. All quantitative traces for this study were injected at the Eclipse Road karst window. The total mass injected for each trace was dependent on the instantaneous discharge at Royal Spring so that coloration of the Georgetown drinking water supply was minimized. The technique is simple and is based on the assumption that the total mass of dye passing the injection point will be the same at the recovery point. Over short distances and with the fast velocities commonly found in karst, this assumption is sufficiently accurate. The spike injection method was not an option for measuring the discharge because the cross-sectional area of the conduit was not accurately known. The mass flux into the system can be expressed as follows:

$$\text{Equation 1. } \frac{C_I}{Q_I} = \frac{Q_R}{C_R}$$

where: C_I is concentration as mass per liter

Q_I is inflow rate in liters per second

C_R is concentration of recovered tracer as mass/liter

Q_R discharge at the recovery site is the unknown variable.



Figure 2. Date and time of samples collected from the cave were recorded for quantitative groundwater tracing.

Table 1. Summary of data from seven quantitative traces made from the Eclipse karst window to the KWIS station in the Royal Spring conduit at the Kentucky Horse Park.

Date and Time	MMcB Velocity at COM Fluor, m/sec	Fluorescein Spike Injection Dye Velocity, m/sec	Stage in Well 24 at COM for Fluorescein Meters above 800 ft	QT TV Method: Tank Volume Based Discharge Calculation, m ³ /sec	QT PR Method: Pumping Rate and Duration, Based Discharge Calculation, m ³ /sec	QT TV Cross-Sectional Area; Discharge/MMcB vel, m ²	QT PR Cross-Section Area: Discharge/(Rate X Duration), m ²	Discharge Using Average Area from Tank Volume and MMcB Velocity, m ³ /sec
12/6/11 14:50	0.28	0.21	6.56	1.82	0.62	6.26	6.44	1.23
3/1/12 14:00	0.24	0.10	3.25	1.68	1.40	6.98	9.21	0.59
12/10/12 15:40	0.31	0.20	4.21	1.03	0.18	3.33	0.88	1.17
2/7/13 18:00	0.23	0.09	2.38	2.39	2.90	10.38	16.66	0.53
3/12/13 13:20	0.33	0.12	3.82	1.60	1.47	4.85	12.35	0.70
3/19/13 11:35	0.39	0.18	5.23	2.24	3.18	5.74	20.05	1.06
4/12/13 15:30	0.26	0.09	1.12	0.91	0.16	3.50	0.96	0.53
Average	0.29	0.14	3.80	1.67	1.42	5.86	9.51	0.83

Abbreviations used in Table 1: COM - center of mass; MMcB - Marsh-McBirney electromagnetic flow velocity meter; QT - quantitative dye tracing technique; TV - volume of reservoir used for tracer dilution for flux calculations; PR - pumping rate for diluted tracer into injections multiplied by the duration in seconds.

By diluting the dye, it was easier to keep the mass flux at a constant rate and to lessen the chance of a small disturbance to the equipment causing a major change in the injection rate of the diluted tracer than to attempt to pump a concentrated solution. The tracer was diluted to 120 liters of water. Water was hauled to the site during the drought; otherwise, the water was obtained from the karst window. A peristaltic pump was used to inject the tracer through a silicon hose. The pumping rate was verified with plain water prior to beginning the trace by timing the filling of a 1-L graduated cylinder. During the trace, the pump ran continuously until the diluted dye was exhausted (typically an hour and a half). The beginning and ending of the injection period was noted. After the midpoint in the injection period but before injection was completed, a grab sample of the diluted tracer was collected and a spike of fluorescein was injected. The concentration of tracer in the mixing tub was known from the volume of water and

mass of dye. However, by sampling the dye stream and measuring the rate and timing of the dye injection, a second injection flux could be independently calculated. We also estimated the cross-sectional area of the cave at the KWIS by three other methods (Table 2): well spacing, video, and Doppler sonar.

Table 2. Summary of cave dimensions measured by various methods at the Kentucky Horse Park KWIS

Well or Calculation	Height 1: Visible Scale, m	Height 2: Video Recorder Depth, m	Width 1: Visible Scale, m	Width 2: By Doppler Sonar, m	Width 3: Distance Measured Between Wells 23 and 24, m	Area 1*: Quantitative Dye Tracing, m²
KyHP20	NA	0.76	NA	7.6		
KhHP23	NA	0.85	NA	NA		
KyHP25	0.86 m	0.9	6 m +/- 0.5	NA		
Average		0.85	6	7.6	5.4	5.89
Areas: width X height, m ²			5.2	6.5	4.6	5.89
Overall cross-sectional area, m ²	N = 4					5.54
*Discharge measured by dye dilution and velocity by Marsh McBirney 201-D magnetic induction meter. The total area is simply the discharge divided by the velocity. The area may include flanking smaller conduits.						

The concentration of fluorescent dye was determined in water samples collected at 10-min increments during the period the dye was expected to pass the KWIS station. The submersible pump was run continuously during the trace and the ISCO periodically collected the sample from the flow through cell. Tracer analysis was done with a Cary Eclipse Varian® fluorescence scanning spectrophotometer. The spectrophotometer response was calibrated with a dilution series of the same tracer used for the experiment.

The exact route of the cave is unknown but it was estimated by using the few available constraints on the cave location (tracer injection and recovery sites, and some association with sinkholes). The length of the flow route was measured with a GIS application found on the KGS

Web site. The route is thought to trend as a tributary from the Eclipse karst window to the southwest. The tributary is confluent with the west—northwest— trending main stem. The flow route in the cave continues to the west for a few hundred meters, but abruptly turns north about a kilometer east of Interstate 75, to where the cave is intersected by the KWIS site. The sum of the three segments was 995 m. This value was multiplied by a 1.1:1 sinuosity ratio for Inner Bluegrass caves (Currens and Graham, 1993) for a total flow path length of 1,095 m. Shorter and longer flow routes can be assumed, but the velocity change is minor within the realistic possible range.

A point velocity was measured for most of the traces at the KWIS site with a Marsh-McBirney 201-D electromagnetic flow meter. The sensor is normally installed near the cave ceiling and is about 1/3 of the cave width off center. The Marsh-McBirney 201-D velocity is therefore unlikely to represent the mean velocity, but is otherwise the only data available for estimating the velocity at the station.

The stage in well 24 has proven reliable and the data record is nearly 100 percent complete. In contrast, the Marsh-McBirney velocity meter has been inoperable a significant percentage of the record at the KWIS station. Thus, there is a strong motivation to use the stage data as a surrogate for the discharge. The datum for the stage recorder is elevation 800 ft and the stage is reported as meters above this datum. Well 24 missed the cave by approximately 0.5 m but responds quickly to high-flow conditions. Down hole video in well 24 and in a well into the cave suggests that fractured rock separating the wells has isolated well 24 from the velocity component of the total head by sheltering the drill hole. Stage in Well 24 can only respond to elevation head and the pressure head on the system.

Although it is recommended that preliminary tests be conducted to assure the complete mixing of the tracer uniformly across the stream or river (Kilpatrick and Wilson, 1989), this was not possible for this project because the flow was inaccessible. Furthermore, conditions such as the length of the flow path and the positioning of the sampling equipment could not be modified even if the mixing was found to be less than ideal.

Principal Findings and Significance

A rating equation was successfully developed by correlating the stage in well 24 to the discharge determined by the quantitative traces. By relating the two values directly any other conditions in the cave that affected discharge were by definition included in the rating equation. Figure 3 illustrates the relationship between the dye-trace-determined discharge and the water level in the adjacent well 24. We also calculated the cross sectional area by dividing the discharge from each Rhodamine WT trace by the Marsh-McBirney velocity. The cross-sectional areas were averaged and the average area was multiplied by the velocity to once again yield a discharge. Because the dye travels along the cave passage for a comparatively long distance

(compared to the equipment spacing), its velocity is affected by changes in the passage cross-sectional area, bends and turns, the roughness of the walls, and fallen stone on the floor, particularly near blockages.

This project resulted in the development of the rating curve needed to calculate discharge through the Royal Spring Cave. The discharge data correlated adequately with the stage record. Without this calculation, the flux of contaminants in the cave would be difficult to quantify. In addition to the constant rate injection used to determine the discharge, we also used spike injections of sodium fluorescein to measure velocity between the two locations. The quantitative groundwater traces conducted at the Kentucky Horse Park were critical for determining the discharge and cross-sectional area of the cave at the KWIS site.

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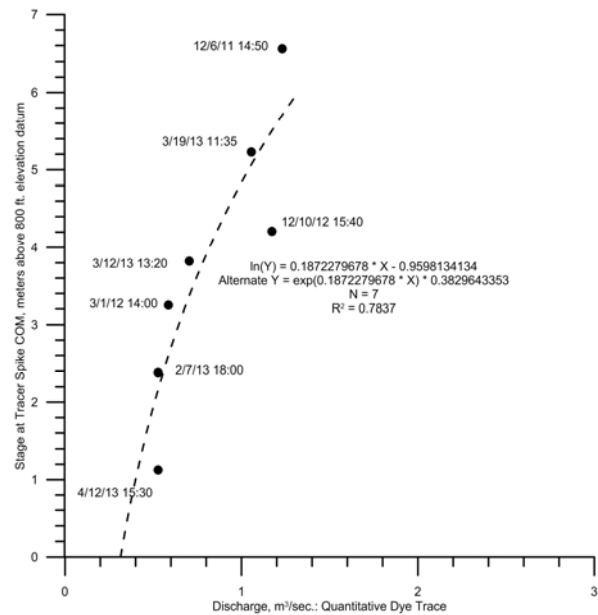


Figure 3. Rating curve for the KWIS station at the Kentucky Horse Park. Relationship of stage in well 24 (meters above 800 ft elevation) to the discharge as determined by quantitative dye tracing.

Information Transfer Program Introduction

Information transfer activities are an important part of the overall program of the Kentucky Water Resources Research Institute. There are two main components, an annual symposium and the institute web sites. The institute also participates in and supports numerous other technology and information transfer activities throughout the year.

Kentucky information transfer activities

Basic Information

Title:	Kentucky information transfer activities
Project Number:	2012KY211B
Start Date:	3/1/2012
End Date:	2/1/2013
Funding Source:	104B
Congressional District:	KY 6th
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	symposium, conferences, newsletter, web site
Principal Investigators:	Lindell Ormsbee

Publications

1. 2013, Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, March 18, 2013, 106 p
2. 2012, Center of Excellence for Watershed Management Watershed Summit Summary Report, September 5, 2012, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, 45 p
3. 2012, Kentucky Stormwater Association 2012 Annual Conference, August 8-10, 2012, Florence, KY, 10 p (www.kystormwater.org)

Kentucky Information Transfer Activities (2012KY211B)

Problem and Objectives

The Water Resources Research Act requires that Institutes or Centers shall:

- (1) plan, conduct, or otherwise arrange for competent applied and peer reviewed research that fosters –
 - (A) improvements in water supply reliability;
 - (B) the exploration of new ideas that –
 - (i) address water problems; or
 - (ii) expand understanding of water and water-related phenomena;
 - (C) the entry of new research scientists, engineers, and technicians into water resources fields; and
 - (D) the dissemination of research results to water managers and the public.
- (2) cooperate closely with other colleges and universities in the State that have demonstrated capabilities for research, information dissemination, and graduate training in order to develop a statewide program designed to resolve State and regional water and related land problems.

Each institute shall also cooperate closely with other institutes and other organizations in the region to increase the effectiveness of the institutes and for the purpose of promoting regional coordination.

Kentucky information transfer activities are conducted in support of these objectives.

Methodology

Information transfer activities are an important part of the overall program of the Kentucky Water Resources Research Institute. There are two main components, an annual symposium and the institute web site (including the electronic newsletter). The Institute also participates in and supports other technology and information transfer activities throughout the year.

The Associate Director develops the program for the Annual Water Resources Symposium. Presentations in both platform and poster format allow for researchers and practitioners to share progress on planned, ongoing, and completed water-related activities throughout the Commonwealth each year.

The Information Specialist Senior assists with creating program announcements and the proceedings volume for the symposium. She also prepares information for the electronic newsletter. She develops and maintains content for several web sites including the main Institute page at: www.uky.edu/WaterResources/. Links for additional sites describing projects and activities (for example volunteer sampling results and watershed

pages for the Kentucky River basin) are provided on the main web site. Research translation to make results accessible for a variety of audiences is a major goal for all of the technology transfer activities of the unit.

The Institute cooperates closely with other groups and agencies in planning additional technology transfer activities in the Commonwealth. These efforts included support for seminar/lectures, other web sites, an open house during Earth Science Week. Institute staff members serve a variety of support roles on technical committees and advisory panels for agencies and volunteer organizations to help disseminate relevant information about ongoing activities and research results;

Principal Accomplishments and Activities

The Kentucky Water Resources Research Institute at the University of Kentucky was designated as a Center of Excellence for Watershed Management by Region IV of the U.S. Environmental Protection Agency on March 21, 2011. With this designation, KWRRI became the first such Center of Excellence in Kentucky and the seventh in the Southeast. These centers help communities identify watershed-based problems and assist with the development and implementation of locally sustainable solutions. To become a Center of Excellence, an institution must demonstrate technical expertise in identifying and addressing watershed needs; involvement of students, staff, and faculty in watershed research; the capability to involve the full suite of disciplines needed for all aspects of watershed management; and a willingness to partner with other institutions. During 2012, KWRRI continued fostering partnerships with a variety of other programs and institutions by hosting a statewide stakeholder meeting targeted at in-state universities, state and federal agencies, and non-governmental organizations active in the Commonwealth on September 5 in Lexington.

Kentucky Water Awareness Month is an educational program of the University of Kentucky Cooperative Extension Service, Environmental and Natural Resources Issues Task Force (the Associate Director of KWRRI is a member). The program promotes overall water awareness for citizens of Kentucky during May each year. Materials are developed by a committee at the state level and distributed to all of the 120 county extension offices in the state. Individual county agents are encouraged to tailor the program to fit their county's specific needs and to use the materials to enhance their program efforts. The materials remain available throughout the year for use by classroom teachers, 4-H volunteers, and others interested in water issues through the ENRI internet site: www.ca.uky.edu/enri/

The Kentucky Stormwater Association Annual Conference was held August 8-10, 2012 in Florence, Kentucky. Through a grant obtained from the Kentucky Division of Compliance Assistance of the Department for Environmental Protection, the KWRRI was able to underwrite the registration fee for one representative from each Municipal Separate Storm Sewer System (MS4) community in Kentucky to attend the annual KSA conference. KWRRI also sponsored an MS4 Monitoring Plan Workshop on November

30, 2012 in Frankfort Kentucky attended by representatives from numerous MS4 communities, their consultants, and agency personnel.

The Kentucky Water Resources Research Institute and the University of Kentucky Department of Earth and Environmental Sciences co-sponsored the National Ground Water Association Henry Darcy Distinguished Lecture "Managing Water Beneath the Agricultural Landscape" by Dr. David Rudolph on April 10, 2013. In the past, the lecture has typically be held in the fall, but this year the speaker's schedule postponed the presentation until spring.

An open house was held on Wednesday evening 10/17/2012 in association with Earth Science Week. This event was co-sponsored with the Kentucky Geological Survey. KWRRI staffed a water exhibit for the elementary, middle school, and high school students and their parents who attended the event (approximately 200 people).

Cyberseminars provided through the Consortium for the Advancement of Hydrologic Sciences, Inc. were made available on the University of Kentucky campus for interested faculty, staff, students, and local professionals. The initial University of Kentucky membership in CUAHSI was underwritten by the KWRRI.

The Kentucky Water Resources Annual Symposium was held on March 18, 2013. Although the date of the symposium fell outside of FY2012, most of the planning and preparation occurred during the fiscal year. Two concurrent sessions provided time slots for 36 oral platform presentations. Twenty posters were also presented during a separate poster session. Approximately 130 people attended the meeting. Abstracts for all of the presentations were distributed to participants on the day of the meeting: Proceedings of the Kentucky Water Resources Annual Symposium, 2013, Kentucky Water Resources Research Institute, Lexington, Kentucky, 106 p. The full proceedings document is also available online through a link on the institute web site. The document includes contact information for all authors and presenters and an abstract for each presentation. Symposium participants also receive a list of attendees providing basic contact information for each individual who pre-registered for the symposium. Attendees include researchers, personnel from local, state, and federal agencies, undergraduate and graduate students, participants from volunteer groups and NGOs, and members of the general public. Conference registration fees are kept low through partial subsidy of symposium expenses (using 104(b) technology transfer and matching funds) to ensure accessibility to individuals from all potential audiences.

Maintenance of the institute web site provides open access for those interested in the activities of the Institute as well as providing links to related sites and information maintained by others. Creation and maintenance of the web site are ongoing throughout the year. Links on the site provide direct access to the Association of State Dam Safety Officials, the Kentucky Research Consortium for Energy and the Environment, the Kentucky River Watershed Watch Sampling Database, the National Institutes for Water Resources, PRIDE, the UK Superfund Basic Research Program Research Translation

Core, the Environmental Research and Training Laboratory, the Kentucky Geological Survey, the Tracy Farmer Center of Sustainable Environment, and the Kentucky Center of Excellence for Watershed Management. The Institute's newsletter WATERWORKS is also available in electronic format through a link on www.uky.edu/WaterResources/

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	13	0	0	0	13
Masters	14	0	0	0	14
Ph.D.	2	0	0	0	2
Post-Doc.	0	0	0	0	0
Total	29	0	0	0	29

Notable Awards and Achievements

2012KY198B - Eastern Kentucky University awarded funds for a second year of post-restoration data collection at Slabcamp Creek and White Pine Branch. The funding (\$2,461) provides support for travel costs, supplies, and hourly wages for undergraduate students through December 2013.

2012KY200B - John McMaine received an award as the Outstanding Graduate Student Oral Presentation at the 2013 Student Water Conference held at Oklahoma State University in April 2013 for his talk entitled: Evaluating the Hydrologic Effectiveness of a Rain Garden in Central Kentucky.

2012KY203B - Roni Grigsby, Masters of Public Health Student in the College of Health and Human Services, Department of Public Health, Western Kentucky University received an award as the 2013 Outstanding MPH Student in Environmental Health Research (awarded April 4, 2013). In addition, Ethan Givens received an award as the 2013 Outstanding Environmental Health Science undergraduate student. Both were involved in this project. The project has also received follow-on funding from the Western Kentucky University College of Health and Human Services (faculty research grant, awarded April 1, 2013 to September 30, 2014, \$3000) and Western Kentucky University Graduate Studies Support (\$1000) for the period April 15, 2013 to August 31, 2013.

2012KY205B - Gilman Oulette was selected as the 2013 outstanding graduate student both within the Western Kentucky University Department of Geography and Geology and in the Ogden College of Science and Engineering. These awards were given in recognition of his overall achievements as a graduate student (his monitoring work at Crump's Cave over the past several years was noted as a significant aspect of this achievement).

2011KY174B - Matthew Nee was recognized by the Western Kentucky University Office of Sponsored Programs as Outstanding Junior Faculty Investigator for fiscal year 2012 in part because of his work on this project. A Kentucky Space Grant Consortium Research Initiation proposal was submitted based largely on the IR data developed in this project. This proposal received \$22000 in funding through the KY EPSCoR program to continue efforts through the end of 2013 and includes support to work with collaborators at NASA's Jet Propulsion Laboratory in Pasadena, California.

Publications from Prior Years

1. 2010KY137B ("A coupled hydrologic and biogeochemical modeling approach to understand if in-stream depositional zones are a carbon source or sink to the atmosphere") - Conference Proceedings - Ford, William and Jimmy Fox, 2013, Watershed-Scale Model of Carbon and Nitrogen Cycles in Streams, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 63-64
2. 2011KY165B ("Effects of streambed sediments on the fate of selenium in eastern Kentucky watersheds contaminated with surface coal mining operations") - Conference Proceedings - Fisher, Edward and Yi-Tin Wang, 2013, Effects of Streambed Sediments on the Fate of Selenium in Eastern Kentucky Watersheds Contaminated with Surface Coal Mining Operations, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 53
3. 2011KY170B ("Quasi-real time sediment discharge measurements using inexpensive experimental technology") - Conference Proceedings - Stewart, Robert, Jimmy Fox, and Cindy Harnett, 2013, Sensor Network for Suspended Sediment Monitoring, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 65-66
4. 2011KY173B ("The effects of prescribed fire on amphibian diversity") - Conference Proceedings - Knopp, Robert, Howard Whiteman, and Chris Mecklin, 2013, The Effects of Prescribed Fire Restoration on Amphibian and Reptile Diversity, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY, p 45